The Fission Yeast Copper-sensing Transcription Factor Cuf1 Regulates the Copper Transporter Gene Expression through an Ace1/Amt1-like Recognition Sequence*

Jude Beaudoin and Simon Labbé‡
From the Département de Biochimie, Université de Sherbrooke, Sherbrooke, Québec, J1H 5N4, Canada

Received for publication, December 13, 2000, and in revised form, January 11, 2001 Published, JBC Papers in Press, January 26, 2001, DOI 10.1074/jbc.M011256200

The Journal of Biological Chemistry Vol. 276, No. 18, Issue of May 4, pp. 15472–15480, 2001 Printed in U.S.A.

Transcriptional regulation of genes encoding critical components of copper transport is essential for copper homeostasis and growth in yeast. Analysis of regulatory regions in the promoter of the ctr4Δ copper transporter gene in fission yeast Schizosaccharomyces pombe reveals the identity of a conserved copper-signaling element (CuSE), which is recognized by the transcription factor Cuf1. We demonstrate that CuSE is necessary for transcriptional activation in response to copper deprivation conditions. Interestingly, the CuSE element bears a strong sequence similarity to the recognition site, denoted MRE (metal regulatory element), which is recognized by a distinct class of copper sensors required for copper detoxification, including Ace1 from Saccharomyces cerevisiae and Amt1 from Candida glabrata.

When a consensus MRE from S. cerevisiae is introduced into S. pombe, transcription is induced by copper deprivation in a Cuf1-dependent manner, similar to regulation by Mac1, the nuclear sensor for regulating the expression of genes encoding components involved in copper transport in S. cerevisiae. UV-cross-linking experiments show that the Cuf1 protein directly binds the CuSE. These results demonstrate that the Cuf1 nutritional copper-sensing factor possesses a module that functions similarly to domains found in the Ace1/Amt1 class of metalloregulatory factors, which allows the protein to act through a closely related MRE-like sequence to regulate copper transport gene expression in S. pombe.

Aerobic life requires trace amounts of transition elements like copper, which have the property to reversibly gain and lose electrons, thereby serving as catalytic centers of numerous proteins involved in a variety of critical enzymatic processes (1–3). Owing to its proclivity to engage in redox reactions, excess copper results in the production of detrimental hydroxyl radicals, which are extremely toxic to cells, causing lipid peroxidation, protein denaturation, and nucleic acid cleavage (4). Therefore, specialized proteins have evolved for the sensing, transport, and sequestration of copper within cells to maintain the delicate balance between essential and toxic levels (5–7).

Three genes in the bakers’ yeast Saccharomyces cerevisiae are induced in response to copper excess: CUP1- and CRS5-encoded metallothioneins and SOD1-encoded Cu/Zn superoxide dismutase (8–10). Metallothioneins are known to counteract metal cytotoxicity by sequestration of cytosolic copper (11, 12). Increased synthesis of these three proteins in response to copper is controlled at the transcriptional level by the Ace1 copper metalloregulatory transcription factor (MRTF) (13–15). Furthermore, the promoter element necessary for copper-inducible transcription of CUP1, CRS5, and SOD1 is denoted metal regulatory element (MRE) and is defined by the consensus sequence, 5′-HTHHNGCTGD-3′ (D = A, G, or T; H = A, C, or T; N = any residue). The GCTG region is termed the core sequence, whereas the region 5′ to the core is known as the T-rich element (16, 55). The core sequence of the MRE is recognized by Ace1 in the major groove, whereas the AT-rich 5′ region is contacted in the minor groove (16, 17). The first subdomain (residues 1–40) (denoted Zn2+ module) of the DNA binding domain of Ace1 was found to bind a single Zn2+ atom (18). The second subdomain of the DNA binding domain (residues 41–110), named copper-regulatory domain (17), harbors 9 Cys residues found as follows: one Cys-X2-Cys motif, three Cys-X-Cys motifs, and a single Cys residue. All of these Cys residues are necessary for copper response, except for the last, Cys105 (17). The arrangement of the Cys residues is predicted to coordinate four Cu2+ atoms through cysteine sulfur bonds (19). Upon copper activation, it appears that the formation of a tetra-copper cluster within the copper regulatory domain via cysteinyl thiolates is critical to make an active detoxifying factor, thereby making the cupro-Ace1 able to interact to MREs in a copper and conformational changes-dependent manner (11, 17–20). The Candida glabrata ACE1 gene ortholog, AMT1, binds MREs with similar motifs and is essential for copper resistance (21, 22).

A copper biochemical pathway distinct from the detoxification system exists to allow S. cerevisiae cells to acquire trace amounts of copper from the environment (2, 5, 23). Yeast genetic studies have led to the identification of the FRE1 (24), CTR1 (25), and CTR3 (26) genes, which encode components involved in the high affinity copper uptake process. When grown under copper starvation conditions, the Fre1 plasma membrane protein reduces Cu(II) to Cu(I) (27), allowing copper ions to be recognized and transported into the cell by two separate, high affinity transport proteins, Ctr1 (28) and Ctr3 (29). A hallmark of these genes is the fact that they are transcriptionally regulated in response to copper.

* This study was supported by Medical Research Council of Canada Grant MOP-42406 (to S. L.) Infrastructure equipment essential for conducting this investigation was obtained through Canada Foundation for Innovation Grant NOF-3754 (to S. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ A Fonds de la Recherche en Santé du Québec Scholar. To whom correspondence should be addressed: Tel.: 819-820-6868 (ext. 15460) or 819-564-5281; Fax: 819-564-5340; E-mail: slabbe@courrier.usher.ca.

† The abbreviations used are: MRTF, metalloregulatory transcription factor; BCS, bathocuproinedisulfonate; bp, base pair(s); BrdU, 5-bromo-2′-deoxyuridine triphosphate; Ctr, copper transporter; CuF1, copper factor 1; CuRE, copper response element; CuSE, copper-signaling element; MRE, metal regulatory element; ORF, open reading frame; SOD, superoxide dismutase; MES, 2-N-morpholinoethanesulfonic acid; PCR, polymerase chain reaction.
scriptionally expressed according to copper need; the transcription of FRED1, CTR1, and CTR3 is up-regulated under copper starvation conditions. This process is under the control of Mac1, another copper-sensing transcription factor harboring homology with the amino-terminal 40 amino acids found in the Ace1/Amt1 copper-activated metalloregulatory proteins but little homology outside of this region (30–32). The carboxy-terminal region of Mac1 harbors two Cys-rich repeats, REP-I and REP-II (also identified as C1 and C2) (39–41). REP-I has been identified as C1 and C2 (39–41). REP-I has been shown to function in copper sensing (31, 39, 41). The copper-specific transcriptional regulation by Mac1 is mediated through cis-acting promoter elements, denoted CuREs (Cu-response elements) with the consensus sequence 5′-TTTGCC(T/G)G(C/A)(G)-3′ (32, 33). Under low copper conditions, Mac1 binds to CuREs as a dimer (34, 35) by making contacts in both the major (with 5′-GC(T/C)/T/C(A/G)-3′) and minor (with 5′-TTT-3′) sequence (36). Interestingly, it has been shown that Cu1+ ions can bind directly to Mac1 (37). Under elevated copper concentrations, Mac1 is released from the CuREs in vivo (32), and it is suggested that it undergoes intramolecular conformational changes to inactive its transcription-activation domain (35, 37, 38).

The fission yeast Schizosaccharomyces pombe exhibits several features, such as genome organization, transcription initiation, signal transduction, post-translational modification, and cell division processing, which are more similar to those from mammalian cells than those of baker’s yeast S. cerevisiae (44). Recent studies of copper homeostasis in S. pombe have identified a high affinity copper transporter, denoted Ctr4, that exhibits more overall sequence homology to human Ctr1 than to S. cerevisiae Ctr1 (6, 45, 46). Likewise, the S. pombe high affinity copper transporter gene ctr4 is transcriptionally regulated by copper availability via the Cuf1 copper-sensing transcription factor (46). Cuf1 activates ctr4 gene expression under copper starvation conditions (46). Therefore, both the Ctr4 copper transporter and the Cuf1 nuclear copper-sensing transcription factor are essential for fission yeast cells to use copper ions from the environment. Although the Cuf1 protein is required for S. pombe high affinity copper transport, Cuf1 displays at its amino terminus an extended homology (amino acid residues 1–61) to the amino-terminal 63 and 62 amino acids of the S. cerevisiae Ace1 and C. glabrata Amt1 class of transcription factors and much less similarity to Mac1, its functional ortholog (46). Cuf1 possesses a cysteine-rich domain at its carboxyl terminus containing five Cys and one His residue that is absent in Ace1/Amt1 but found duplicated in both Mac1 and Grisea of Podospora anserina (43, 46). With respect to copper detoxification in S. pombe, the only known molecules for sequestering excess of copper ions are the phytochelatins, since the fission yeast lacks metallothioneins (42). No precise molecular mechanism of how copper sequestration is regulated in S. pombe has yet been identified.

In this study, we identify a cis-acting element found upstream of the copper transport genes in S. pombe termed copper-signaling element (CuSE) that is necessary for activation in response to copper deprivation conditions. The CuSE sequence bears a strong sequence similarity to the MRE for the Ace1 and Amt1 copper metalloregulatory detoxifying transcription factors from the bakers’ yeast (S. cerevisiae) and the yeast C. glabrata. When MREs from S. cerevisiae are expressed in the fission yeast S. pombe, transcriptional regulation occurs in the opposite direction from that observed for the S. cerevisiae CUP1, SOD1, and CBS5 genes, which are activated in an MRE-dependent manner in response to copper. Moreover, the transcriptional copper starvation-mediated activation that is observed when MREs of S. cerevisiae are introduced in S. pombe depends on Cuf1. UV-cross-linking experiments using extracts derived from a heterologous system expressing a functional cuf1 gene reveal that the Cuf1 protein binds the CuSE element. Taken together, these results show that the S. pombe Cuf1 nutritional copper-sensing factor acts through a closely related MRE-like element to regulate expression of fission yeast genes encoding components of the high affinity copper transport machinery.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions**—The S. pombe strains used in this study were the wild-type FY254 (h− can1-1 leu1-32 ade6-M210 ura4-D18), the cuf1Δ disruption strain (h− can1-1 leu1-32 ade6-M210 ura4-D18 cuf1Δ::ura4−), and the ctra4 disruption strain (h− can1-1 leu1-32 ade6-M210 ura4-D18 ctra4Δ::ura4−), which were described elsewhere in detail (46). For ectopic expression of the cuf1 gene in S. cerevisiae, the ace1 strain, denoted DTY59 (MATa his6 leu2-3,112 ura3-52 ace1− D225 CUP1-R3) (47), was utilized to ensure the presence of Cuf1 as sole protein with the ability of interacting with MREs. Under nonselective conditions, S. pombe cells were grown in yeast extract plus supplements (225 mg/liter adenine, histidine, leucine, uracil, and lysine). When plasmid maintenance was required, Edinburgh minimal medium was used as selective medium (48). S. cerevisiae strains were grown either in yeast extract/petone/dextrose medium or in the appropriate drop-out synthetic media (49). To assess the role of Cuf1 in S. cerevisiae, the cells were grown in a modified minimal medium containing 0.67% yeast nitrogen base minus copper and iron, 2% dextrose, 50 mM MES buffer (pH 6.1), and 10 μM NH4Fe(SO4)2 as described previously (32).

**Promoter Deletions and Site-directed Mutagenesis**—The plasmid pSP1ctr4-737 carries a 1607-bp Xho1-BamHI PCR-amplified fragment, which harbors the ctdrv− promoter region up to −737 from the start codon of the ctdrv gene in addition to the gene itself. This pSP1 (52) derivative was digested with BamHI (accessible end) and SacI (protected end) to perform unidirectional nested 5′ deletions of the ctdrv− promoter. The plasmid pSP2ctr4-213 containing nucleotides from position −213 to position −524 with respect to the ATG of the ctdrv− ORF was created to introduce mutations in the CuSEs by site-directed mutagenesis. Precisely, the oligonucleotides 5′-CCTGTC-CTTAAAACTTACATTCTTTACTGCAATTAATCCCTGATTG-3′ (and 5′-GAAGTCCGAAATTCTCTATTCCAAACAGGTCGAGATTTC-3′) (letters that are underlined represent multiple point mutations in the CuSEs) were used either together or separately in conjunction with the pSP2ctr4-213 and the Cuf1 nutritional copper-sensing factor acts through a closely related MRE-like element to regulate expression of fission yeast genes encoding components of the high affinity copper transport machinery.
FLAG\textsubscript{low} (5'-GGCCGCTTTTGTCATCGCTCTTGATGCGCCTTGTGCTATCGTGGCTTTGACTC-3'), encoding two copies of the GPD vector. These purified oligonucleotides were annealed pairwise to form double-stranded DNA with E61Gt1 overhangs and then ligated into the NotI site of pSKcuf1' -Not1 in which a NotI restriction site was previously engineered by PCR and placed in-frame just before the stop codon of the cuf1' gene (46). Subsequently, the cuf1' FLAG2 was isolated from this pSK derivative with EcoRI and BamHI and then cloned into the pGEM-T-Zfr (+) vector (Promega, WI). To release the cuf1' FLAG2 gene from this pGEM derivative, both XbaI and BamHI were used, allowing unidirectional gene insertion into the SpeI (which produces compatible ends to that of XbaI) and BamHI sites of the p416GPD/p426GPD vector for ectopic expression in S. cerevisiae cells (51). For immunoblot analysis, S. cerevisiae DTY59 cells were transformed with p416GPD (as control) and p416PDcuF1/FLAG2. Yeast cells were grown to A\textsubscript{600} = 1.1 either with or without 10 \textmu M copper chelator BCS and with or without 100 \textmu M CuSO\textsubscript{4}. Total cell lysates were prepared by glass bead disruption in lysis buffer (20 mM HEPES (pH 7.9), 10% glycerol, 75 mM NaCl, 1 mM EDTA, 1 mM phenylmethysulfonyl fluoride, 2 \mu g/ml aprotinin, 1 \mu g/ml peptatin A, and 0.5 \mu g/ml leupeptin). The cells were lysed by vortexing for 1 min at top speed 5 times with 1–2 min intervals on ice. After centrifugation for 6 min at 4,800 rpm, the protein concentration of the supernatant was determined by the Bradford assay. Equal amounts of each sample were used to detect the Cuf1FLAG2 protein using anti-FLAG M2 antibody (Eastman Kodak Co.) as described by the manufacturer. To carry out immunodetection of the 3-phosphoglycerate kinase, monoclonal anti-PGK antibody 22C5-D8 (Molecular Probes, OR) was used. When examined in nodetection of the 3-phosphoglycerate kinase, monoclonal anti-PGK antibody 22C5-D8 (Molecular Probes, OR) was used. When examined in...
the three elements is sufficient to confer regulation in response to copper levels (Fig. 2). As compared with the wild type promoter segment, ~70 and 60% of the response was still observed when either the first element or the two other elements were unaltered, respectively, suggesting that the first element (positions −721 to −712) is slightly favored for copper starvation induction. Although a low basal level of expression was observed when all three elements were mutated, there was a complete lack of either down or up-regulation of the ctr4+ gene. Furthermore, deletion of 24 bp (from −737 to −713) of the ctr4+ promoter already harboring mutated elements abolished even the low basal gene expression of ctr4+ (Fig. 2). Taken together, these data show that a conserved element in the ctr4+ promoter, which we term CuSE, with the sequence 5′-D(T/A)DDHGCTGD-3′ (D = A, G, or T; H = A, C, or T) is required, at least in a single copy, for both repression of basal expression and activation of copper ions and activation under copper starvation conditions.

The CuSEs Confer Copper Responsiveness to the Minimal Promoter CYC1 Fused to lacZ—To examine whether the CuSEs could regulate a heterologous reporter gene in a copper-dependent manner, the ctr4+ promoter segment of 213-bp (positions −737 to −524) was inserted into the minimal promoter of the CYC1 gene fused to lacZ (Fig. 3). In the presence of copper ions, this fusion promoter gene was repressed by −9–12-fold. Conversely, under copper starvation conditions, lacZ mRNA expression was induced (~3-fold). Interestingly, CYC1-lacZ fusion genes containing the previously described CuSE mutants (Fig. 3) derived from the ctr4+ promoter repress lacZ mRNA with an overall efficiency similar to that observed in the endogenous ctr4+ gene, except for the magnitude of the response, which is more pronounced when a heterologous system is used. Importantly, the sequence 5′-D(T/A)DDHGCTGD-3′ (D = A, G, or T; H = A, C, or T) was also found in multiple copies in the ctr5+ promoter, which drives the expression of a novel gene encoding an indispensable partner of the Ctr4 copper transporter involved in high affinity copper uptake in S. pombe.2 As illustrated in Fig. 4, fusion of short regions containing 175 bp from the ctr4+ or ctr5+ promoters encompassing CuSE elements were able to copper-regulate expression of lacZ mRNA. Although the ctr4+ promoter harboring this shorter fragment (positions −737 to −562) of 175 bp is regulated in a copper-dependent manner, the overall magnitude of the response decreases by ~70% as compared with the longer fragment (positions −737 to −524) of 213 bp. For both promoter fusions, ctr4+::CYC1-lacZ and ctr5+::CYC1-lacZ, the integrity of the CuSEs is essential for copper-responsive gene expression since CuSE mutants abrogate any regulation by copper (Fig. 4).

Therefore, these results suggest that the S. pombe copper transport genes share a common promoter element, denoted CuSE (5′-D(T/A)DDHGCTGD-3′), which is necessary for activation in response to copper limitation.

Cuf1 Acts through Metal Regulatory Elements—Although we have demonstrated previously that Cuf1 regulates the expression of essential copper transport genes in S. pombe, the Cuf1 amino-terminal 61 amino acids more closely resemble aminoterminal domain 63 and 62 amino acids of Ace1 (51%) and Amt1 (45%) copper-detoxifying factors than the corresponding region of Mac1 (39%) (46). Based upon this observation and because of our finding that the CuSE bears a strong sequence similarity to the Ace1 and Amt1 binding sites (MREs), we sought to assess whether MREs of the S. cerevisiae CUP1 gene could serve in S. pombe for gene regulation as a function of copper availability by Cuf1. In the cuf1+ strain, when a 129-bp CUP1 promoter DNA fragment (positions −292 to −163) encompassing four MREs was inserted in its natural orientation into the minimal promoter of the CYC1 gene fused to lacZ, the expression of lacZ mRNA was indeed copper-regulated, exhibiting induction in the presence of the Cu2+ chelator BCS and repression upon the addition of copper (Fig. 5). In the cuf1Δ mutant strain, although a high basal level of lacZ mRNA was detected in the presence of the wild type MREs, perhaps owing to the action of another transcription factor, the steady-state levels of lacZ mRNA were unregulated by modulation of copper status (Fig. 5). Furthermore, for both the wild type and mutant strains, no regulation by copper was observed when the MRE elements were mutated (Fig. 5). Therefore, when MREs from S. cerevisiae are introduced in the fission yeast S. pombe, transcriptional regulation occurs in the opposite direction from that observed for the S. cerevisiae CUP1, SOD1, and CRS5 genes, in which the presence of the MRE fosters the activation of gene expression in response to copper.

Cuf1 Interacts Directly with CuSEs—Based on the gene expression data we obtained, we predicted that the Cuf1 factor

---

2 D. J. Thiele, personal communication.
directly interacts with CuSEs to mediate copper regulation. To test this hypothesis, we generated a Cuf1FLAG2 fusion protein, allowing its detection in DNA-protein complexes using commercially available anti-FLAG M2 antibody. Importantly, to test whether insertion of the FLAG tags interfered with Cuf1 function, the Cuf1FLAG2 fusion protein was tested for its ability to complement the cuf1D nonfermentable carbon source growth defect in S. pombe. The tagged Cuf1 protein fully complemented the respiratory deficiency (data not shown). Furthermore, as assessed by RNase protection experiments when transformed into the cuf1Δ fission yeast strain, the cuf1Δ FLAG2 allele accurately functions to regulate the cuf1Δ gene expression in response to copper ions in a manner indistinguishable from the cuf1+ wild type allele (Fig. 6). To examine whether the Cuf1 protein interacts with CuSE, we used the cuf1Δ FLAG2 fusion gene in a heterologous context as the only S. pombe gene expressed in a bakers’ yeast system (51) lacking the endogenous ACE1 gene. The ace1Δ strain was utilized to...
ensure that Cuf1 was the sole protein with the ability to recognize the DNA probe, which contains a GCTG core with a T located four nucleotides upstream of the first G in the GCTG core. When expressed, the cuf1FLAG2 fusion protein regulates in a proper manner the ctr4+ mRNA levels. Cultures of the cuf1Δ mutant strain transformed with either pSP1 vector or pSP1cuf1FLAG2 were incubated in the absence (−) or presence of CuSO4 (1 and 100 μM) or BCS (B) (100 μM) for 1 h. After treatment at 30 °C, total RNA was isolated. Shown is a representative RNase protection assay of ctr4+ and act1+ mRNA steady-state levels, indicated by arrows, respectively.

FIG. 5. The bakers’ yeast MRE elements serve to down-regulate gene expression in a copper- and Cuf1-dependent manner. A, The isogenic fission yeast strains FY254 (cuf1+) and SPY1 (cuf1Δ), transformed with pCPLEU2CUP1-CYC1-lacZ either with wild type (WT) or mutant MREs, were incubated in the absence (−) or presence of CuSO4 (1 and 100 μM) or BCS (B) (100 μM) for 1 h followed by RNA isolation and analysis. Results illustrated are representative of three independent experiments. B, schematic representation of the two plasmids assayed in the wild type and cuf1Δ mutant strains. The open boxes represent the wild type MREs, and the boxes marked with an × indicate the mutant versions. For each assay, values for fold repression by copper and activation by BCS are shown. The nucleotide numbers refer to the position relative to the A of the ATG codon of the CUP1 ORF.

FIG. 6. The Cuf1FLAG2 fusion protein regulates in a proper manner the ctr4+ mRNA levels. Cultures of the cuf1Δ mutant strain transformed with either pSP1 vector or pSP1cuf1FLAG2 were incubated in the absence (−) or presence of CuSO4 (1 and 100 μM) or BCS (B) (100 μM) for 1 h. After treatment at 30 °C, total RNA was isolated. Shown is a representative RNase protection assay of ctr4+ and act1+ mRNA steady-state levels, indicated by arrows, respectively.

FIG. 7. The CUP1 gene expression is down-regulated in response to copper in ace1A cells expressing S. pombe Cuf1. A, Bakers’ yeast ace1A cells transformed with pGPD418Cuf1FLAG2 were grown to early log phase in a modified SD media containing ~16 μM copper (32). After no treatment (−) or incubation in the presence of either 100 μM CuSO4 or 100 μM BCS (B), protein extracts were prepared from the cells and then analyzed by immunoblotting using either anti-FLAG M2 or anti-PGK (as an internal control) antibody. We note that the tagged Cuf1 migrates at ~54-kDa despite a predicted molecular mass of 45-kDa. B, the ace1Δ strain, transformed with either the low-copy plasmid expressing cuf1FLAG2 or the vector alone, was grown to mid-log phase and treated with CuSO4 (100 μM) or BCS (B) (100 μM) for the indicated time. After total RNA isolation, a representative RNase protection analysis of CUP1 and ACT1 mRNA levels, which exhibits repression of CUP1 mRNA expression in the presence of copper in a Cuf1FLAG2-dependent manner. As a control (right side), the S. cerevisiae isogenic wild type strain (ACE1) displays high steady-state levels of CUP1 mRNA in response to copper. M, reference marker.
affinity copper transport system (46). Indeed, we have shown previously that deletion of the cuf1" - gene results in at least three phenotypes associated with copper starvation in yeast cells: inability to use respiratory carbon sources, impaired superoxide dismutase activity, and defects in iron accumulation (46). All three phenotypes are restored by the addition of exogenous copper to the medium, consistent with cuf1Δ strains being specifically defective in the expression of genes required for high-affinity copper transport. Furthermore, the previous observation that in S. pombe, expression of the high-affinity copper transporter Ctr4 is dependent on the Cuf1 protein defines a critical role for trans-activation of copper transport gene by Cuf1 (46).

To identify the cis-acting elements necessary for copper starvation activation of copper transport gene expression by Cuf1, we have conducted detailed studies on the cuf1- promoter. Although there are two copies of the Mac1 binding site, 5’-TTTGC(T/G)(C/A)(G)-3’, denoted CuRE (32, 33), located between positions −323 and −381 in the cuf1- promoter, no effect on copper-responsive regulation of the cuf1- promoter was observed when these two elements were mutated.3 This latter observation is also consistent with the inability of Cuf1 to suppress a number of copper-remedial phenotypes associated with mac1Δ disruption when the S. pombe cuf1- gene is ectopically expressed in a S. cerevisiae mac1Δ strain.3 Our studies have revealed that the cuf1- and cuf5- promoters are regulated in response to fluctuations in copper concentrations by a consensual cis-acting sequence, 5’-D(T/A)DDHGCCTG-3’ (D = A, G, or T; H = A, C, or T) termed CuSE, which is highly similar to the binding sites for the Ace1 and Amt1 copper MRTFs, MREs. This finding is consistent with the high percentage of homology within residues 1–61 of Cuf1 amino terminus to the MREs. This finding is consistent with the high percentage of homology within residues 1–61 of Cuf1 amino terminus to the Mac1 protein, which requires at least two copies of the CuREs for copper-regulated gene expression (32, 38). Although few

---

3 S. Labbé, unpublished data.
differences are observed between the CuSE and MRE consen-
sus sequences (Fig. 9), the binding sites appear to be inter-
changeable since when MREs from S. cerevisiae are introduced in
the fission yeast S. pombe, Cuf1 can BCS-activate target
gene expression through those MREs (Fig. 5). It is anticipated
that some nucleotides within the CuSE should optimize copper
starvation induction of gene expression. Which nucleotides con-
tribute to the magnitude of this regulatory response must
await a comprehensive dissection of the CuSE. With respect to
the conserved T-positioned four nucleotides upstream of the
GCTG core element (Fig. 9), it has been shown previously (16)
that its substitution by adenine results in ~20% reduction in
DNA binding by Amt1. Based on this observation, we would
predict that the CuSE between positions ~596 and ~581 has a
weaker strength for copper transcriptional regulation.

To investigate further the mechanism by which Cuf1 regu-
lates target gene expression through CuSEs in response to
copper, we have used a S. cerevisiae yeast system (51) in which
the endogenous ACE1 gene was inactivated. By using this
approach, we sought to ensure the presence of Cuf1 as sole
protein with the ability to recognize MRE-like elements. A
heterologous test is essential, because we have identified a
second gene in S. pombe, denoted cuf2+ (SPCC584.02). This
putative MRTF exhibits 41% identity to the amino-terminal 61
amino acids of Cuf1.3 When expressed in S. cerevisiae ace1Δ
conditions in which copper is scarce, the Cuf1 protein
fosters high basal levels of CUP1 mRNA. Conversely, when
S. cerevisiae ace1Δ cells were grown in the presence of copper,
the CUP1 mRNA levels were repressed by Cuf1, con-
sistent with the manner by which Cuf1 regulates copper trans-
port expression in S. pombe. To determine whether Cuf1
binds to CuSE when ectopically expressed in S. cerevisiae ace1Δ
cells, a BrdUrd-double-stranded CuSE oligomer derived from
the ctf4+ promoter (positions ~700 to ~731) was used for
UV-cross-linking analysis (54). Precisely, the BrdUrd
was placed on the noncoding strand two nucleotides upstream of the
GCTG core element (position ~718) since the Cuf1 protein is
predicted to interact with one face of the DNA double helix at
adjacent minor (encompassing that position ~718) and major
(containing the GCTG core element) grooves. Indeed, when the
reactive base (BrdUrd) was positioned into the CuSE, a DNA-
Cuf1-dependent complex was observed (Fig. 8). Interestingly,
this DNA-protein complex of ~64-kDa was only observed when
the extract preparations were obtained from yeast cells grown
under conditions of low copper availability. When cells were
grown in the presence of copper, no such DNA-protein complex
was detected.

How does Cuf1 function in copper deprivation activation
through the Ace1/Amt1-like recognition sequence? It is intriguing
that Cuf1 displays DNA binding specificity similar to that of
S. cerevisiae Ace1 and C. glabrata Amt1, yet clearly this
recognition sequence is used for copper transporter gene ex-
pression in S. pombe. Although the first 61 residues of Cuf1
exhibit a strong homology to this same region of Ace1 and
Amt1, Cuf1 does not possess the second half of the Ace1/Amt1
copper regulatory domain in which two highly conserved Cys-
X-Cys sequence motifs are found (46). These two pairs of Cys
that are missing in Cuf1 make improbable the formation of
Ace1/Amt1-like copper regulatory domain that consists of two
lobes separated by a cleft in which the Cu(S)6 center takes place
in the presence of copper ions (17). Interestingly, Cuf1 harbors
a REP-like motif near its carboxyl terminus, which shows a
high percentage of identity (48%) to a similar domain found in
S. cerevisiae Mac1 (46). Because in Mac1, the REP-I domain is
able to sense copper ions, fostering intramolecular conforma-
tional changes and thereby inactivating the Mac1 DNA binding

and its transactivation function, the possibility exists that the
Cuf1 REP-like domain acts in a similar manner. Therefore,
Cuf1 resembles two distinct types of copper MRTFs, sharing
recognition sequence specificity to bind DNA of the Ace1 and
Amt1 proteins while playing an essential role in the regulation
of the high affinity copper transporter protein gene expression
in S. pombe as found for the Mac1 protein in S. cerevisiae.
47. Butler, G., and Thiele, D. J. (1991) *Mol. Cell. Biol.* **11**, 476–485
48. Alfa, C., Fantes, P., Hyams, J., McLeod, M., and Warbrick, E. (1993) in *Experiments with Fission Yeasts: Laboratory Course Manual*, pp. 1–186, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
49. Kaiser, C., Michaelis, S., and Mitchell, A. (1994) in *Methods in Yeast Genetics*, pp. 1–234, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
50. Iacovoni, J. S., Russell, P., and Gait, F. (1999) *Gene* **232**, 53–58
51. Mumberg, D., Müller, R., and Funk, M. (1995) *Gene* **156**, 119–122
52. Cottarel, G., Beach, D., and Deuschle, U. (1993) *Curr. Genet.* **23**, 547–548
53. Turner, R. B., Smith, D. L., Zawrotny, M. E., Summers, M. F., Posewitz, M. C., and Winge, D. R. (1998) *Nat. Struct. Biol.* **5**, 551–555
54. Lin, S. Y., and Riggs, A. D. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 947–951
55. Zhu, Z., and Thiele, D. J. (1996) *Cell* **87**, 459–470
The Fission Yeast Copper-sensing Transcription Factor Cuf1 Regulates the Copper Transporter Gene Expression through an Ace1/Amt1-like Recognition Sequence
Jude Beaudoin and Simon Labbé

J. Biol. Chem. 2001, 276:15472-15480.
doi: 10.1074/jbc.M011256200 originally published online January 26, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011256200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 33 of which can be accessed free at http://www.jbc.org/content/276/18/15472.full.html#ref-list-1