Histone H2A and Spt10 Cooperate to Regulate Induction and Autoregulation of the CUP1 Metallothionein*

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Copper is an essential cellular cofactor that becomes toxic at high levels. Copper homeostasis is tightly regulated by opposing mechanisms that control copper import, export, and copper binding capacity within the cell. High levels of copper induce the expression of metallothioneins, small sulfhydryl-rich proteins with high metal binding capabilities that serve as neutralizers of toxic levels of metals. In yeast, the CUP1 gene encodes a copper metallothionein that is strongly induced in response to metals and other stress and is subsequently rapidly down-regulated. Activation of CUP1 is mediated by the copper-responsive transcriptional activator Ace1, and also requires the histone acetylase Spt10 for full induction. We have examined the role of histone H2A in the normal regulation of the CUP1 gene. We have shown that specific H2A mutations in combination with spt10 deletions result in aberrant regulation of CUP1 expression. Certain lysine mutations in H2A alleviate the transcriptional defect in spt10Δ strains, though CUP1 activation is still delayed in these mutants; however, CUP1 shutdown is normal. In contrast, serine mutations in H2A prevent CUP1 shutdown when combined with spt10 deletions. In addition, sswi/swnf mutants exhibit both impaired CUP1 induction and failure to shut down CUP1 normally. Finally, different Spt10-dependent histone acetylation events correlate with induction and shutdown. Taken together, these data indicate that CUP1 transcriptional shutdown, like induction, is an active process controlled by the chromatin structure of the gene. These results provide new insights for the role of chromatin structure in metal homeostasis.

All cells must be capable of responding rapidly and specifically to a variety of external stressors, such as sudden changes in temperature, oxygen starvation, DNA-damaging agents, or heavy metals. Metals such as copper, iron, and zinc are essential in enzyme catalysis, protein structure, and respiration. However, these key elements become toxic at high levels. Metal ion homeostasis is tightly controlled, with excess ions exported or sequestered while maintaining sufficient cellular stores to satisfy the cofactor requirements of essential enzymes. Much of this regulation occurs at the level of transcription. The yeast Saccharomyces cerevisiae is a powerful model for understanding cellular mechanisms of metal ion homeostasis (1–3).

All transcription occurs in the context of chromatin, a complex structure in which the DNA is wrapped around histone octamers to form nucleosomes. The nucleosome consists of approximately two turns of DNA wrapped around an octamer of histone proteins. Two copies each of histones H2A, H2B, H3, and H4 comprise the core histone octamer. Each core histone contains an N-terminal domain that extends from the surface of the octamer, histone H2A has an additional C-terminal domain. The histone tails are the targets of the majority of the known post-translational modifications of the core histones. These modifications include lysine acetylation and methylation, serine phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation (4–6).

Tail modifications of histones H3 and H4 have been extensively characterized and have been shown to be critical in multiple cellular processes, particularly in transcription (7–9). In contrast, histone H2B modifications have only recently been implicated clearly in transcription (10–12) and DNA double strand break repair (13), and histone H2A/H2AX modifications have primarily been investigated in the process of DNA double strand break repair (14, 15). In one case yeast H2A modifications have been implicated in transcription: specific H2A modification sites are required for normal gene silencing at telomeres (16). We have investigated the role of the H2A tails in the regulation of the copper-inducible CUP1 gene.

The yeast CUP1 gene encodes a copper metallothionein, which is strongly and rapidly induced in the presence of copper (17). CUP1 is an excellent model for understanding the role of histone modifications in inducible gene expression. The chromatin organization of the CUP1 promoter has been extensively characterized (18, 19). In the uninduced state, nucleosomes are organized into defined clusters of positions. The binding of copper to the activator Ace1p leads to its folding and subsequent binding to UASs in the CUP1 promoter. Ace1p binding results in the repositioning of nucleosomes throughout the CUP1 promoter and open reading frame, potentially via recruitment of a chromatin remodeling enzyme, leading to increased promoter accessibility. Subsequently, histones H3 and H4 are acetylated (20, 21). This acetylation is dependent on the Spt10p acetyltransferase (21). Spt10p is required for maximal levels of CUP1 induction, but CUP1 expression is not eliminated in spt10 deletion strains. One possibility is that Spt10-dependent acetylation is partially redundant with the putative remodeling activity. Alternatively, other histone modifications in addition to Spt10-dependent acetylation may be important for CUP1 activation.

We have investigated the roles of the H2A tails in CUP1 regulation, and have studied the interaction between H2A and Spt10 in regulation of CUP1. We show that N- and C-terminal mutations in H2A impair CUP1 induction in response to copper. Furthermore, H2A C- and N-terminal mutations combined with spt10 deletions result in striking alterations in the kinetics of CUP1 regulation. Specific lysine, serine, and threonine mutants impair and/or delay CUP1 activation to varying degrees in spt10Δ strains. Even more strikingly, particular serine

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mutants prevent normal shutdown of CUP1, suggesting that an active process of autoregulation is perturbed in these strains that may depend on histone phosphorylation. Consistent with this, phosphorylation of H2A Ser-129 is detected at the CUP1 promoter coincident with down-regulation. Finally, CUP1 kinetics are similarly perturbed in a swi1Δ/swi1Δ mutant strain. Taken together, these data indicate that active chromatin modification/remodeling is involved in both the activation and autoregulation of CUP1, facilitating the dynamic control of copper homeostasis.

EXPERIMENTAL PROCEDURES

Yeast Strains, Plasmids, and Knockout Constructs—Most strains used in this study are derived from FY406 (MATa, hta1-1::LEU2, hta2-htb2::TRP1, leu2-1, ura3-52, trpl-63, his3-200) and contain either the wild-type HTA1-HTB1 locus on a CEN-ARS plasmid (URA3, JKY28; or HIS3, JKY38), or a mutant hta1 allele + wild type HTB1 on a CEN-ARS plasmid (HIS3). The swi1Δ strain (JKY20) has the genotype MATa, swi1::LEU2, ey2-801, ade2-101, leu2-1, his3-200, ura3-99. The isogenic wild-type strain for the swi1Δ strain behaved equivalently to the FY406-derived wild-type strain; the wild-type data reported are from the FY406-derived strain.

Mutant hta alleles were generated using the QuikChange™ site-directed mutagenesis kit (Stratagene). Mutant hta alleles are exchanged for the wild-type allele by a plasmid shuffle method, in which the strain carrying the URA3-marked HTA1-HTB1 plasmid (JKY29) is transformed with the HIS3-marked plasmid containing the mutant hta allele. HIS+ transformants that have lost the wild-type HTA1 plasmid are selected for using medium supplemented with 5-FOA, which selects against cells expressing URA3. SPT10 was deleted directly in strains containing hta alleles (HIS3 marked) using a URA3 gene flanked by 50 bp of SPT10 homology. This construct was generated via PCR using the following primers to amplify URA3 from pRS406: 5′-GATGAGTAAAAAGTGTACC GATCAAGAACAACTCTAGACCT-3′ and 5′-TATTAGAATTGTGCTCTG-3′.

CUP1 Induction and Detection—To induce CUP1, yeast strains are grown to mid-log phase (OD600 of 0.5–0.7) in YEPD, then CuSO4 is added to a final concentration of 1 mM. Cells are incubated at 30 °C for 4 h, then harvested, and total RNA is isolated using glass bead lysis. Northern blots are performed by standard methods. The indicated times, then harvested, and total RNA is isolated using glass bead lysis. Northern blots are performed by standard methods.

RESULTS

H2A Tails Are Involved in CUP1 Induction—CUP1 transcription is induced 10–20-fold within 5 min of addition of 1 mM copper. CUP1 subsequently autoregulates its own expression, and transcription levels drop to a much lower steady-state level by 30–40 min after copper exposure (23, 24). We initially measured CUP1 expression at three points: in the absence of copper, at 10 min after addition of 1 mM CuSO4, when expression is maximal for wild type, and at 30 min after addition of CuSO4, when CUP1 is down-regulated. The amount of CUP1 mRNA is detected by Northern blot and quantitated by phosphorimager. All CUP1 expression levels are normalized to expression of ACT1 as a loading control. The average normalized data for at least three independent experiments are presented in each graph, and standard errors are shown.

We have analyzed CUP1 expression in strains containing a variety of alanine substitutions in the H2A C and N termini (Fig. 1). Most single mutations in the H2A C terminus have minimal effects on CUP1 induction at 10 min, though alanine substitutions at lysines 120 or 121 show a 2-fold reduction in CUP1 levels at 10 min (Fig. 1A). However, most C-terminal mutations show a reduction in levels of CUP1 at 30 min after copper addition (Fig. 1A).

Because CUP1 expression changes so rapidly, we confirmed these effects by measuring CUP1 levels every 5 min in several of these strains, in case we had missed a peak of expression. Fig. 1B shows the results of the short time course for two H2A lysine mutants, htaK120A and htaK127A, compared with wild type. CUP1 induction is rapid and strong in the wild-type strain, with strong signal within 5 min of copper addition, reaching maximum induction levels by 10 min, and beginning to decrease by 25–30 min (Fig. 1B, top panel). Both htaK120A (middle panel) and htaK127A (bottom panel) exhibit similar induction kinetics compared with wild type, while showing a CUP1 induction level consistent with that shown in Fig. 1A; i.e., a 2-fold reduction in CUP1 mRNA in the htaK120A strain versus nearly wild-type levels in the htaK127A strain.

We have also examined the effects of mutations in the H2A N terminus on CUP1 expression. This tail appears to play a role in CUP1 induction comparable to the C terminus, in that alanine substitutions at serine and lysine residues in the N terminus result in reduced levels of CUP1 mRNA. Induction data for two of these mutants, htaS2A and htaK5A, are shown in Fig. 1C. Both of these strains exhibit ~40% reduction in CUP1 mRNA levels compared with wild type. Similar results are obtained for other residues in the N terminus (data not shown).

These results indicate that both the N- and C-terminal tails of histone H2A are required for normal induction of CUP1. Because all of the residues chosen are sites of potential modification (lysines, serines, and threonine), an obvious question is whether specific modifications of these residues are involved in CUP1 induction. Indeed, substitution of a glutamine for the lysine at position 127 (a substitution commonly used to mimic lysine acetylation) appears to alleviate the minor CUP1 induction defect of the alanine mutation at this site (data not shown). Since it is already known that modifications of histones H3 and H4 also occur during CUP1 induction (20, 21), it will be interesting to determine whether H2A modifications do occur, and cooperate with H3/H4 modifications at CUP1.

Different Histone Modifications Coincide with CUP1 Induction and Autoregulation—Previous work has shown that the Spt10 histone acetyltransferase is required for normal induction of CUP1 (21). spt10Δ mutants exhibit delayed induction, reduced levels of CUP1 mRNA, and sensitivity to high levels of copper. These mutants also result in loss of acetylation of histones H3 and H4 at CUP1. We wished to understand the different contributions of Spt10 and H2A to CUP1 regulation. To address this, we knocked out the SPT10 gene in strains containing different mutations in H2A. In looking at genetic interactions between H2A and SPT10, it is important to note that Spt10 itself is involved in transcription of certain histone genes (25, 26). Spt10 is required for expression of the HTA2-HTB2 locus; fortunately, our mutant histones are expressed solely from HTA1 promoters, and their expression is not impacted by the deletion of SPT10.

We first compared the expression of CUP1 in spt10Δ cells in the presence of wild-type H2A. Consistent with previous reports (21), we observe both delayed induction and significantly reduced levels of CUP1 mRNA in the absence of Spt10 (Fig. 2A).

It has been shown that histones are acetylated at CUP1 upon exposure to copper (20, 21), and that this acetylation requires Spt10 (21). However, in another study, AceI-dependent CUP1
induction resulted in no change in H3 acetylation and a decrease in H4 acetylation (27). These differences could be attributable to different strain backgrounds used in these studies. However, another possibility is that histone modifications at CUP1 are as dynamic as CUP1 expression itself, and these different studies were measuring histone acetylation at different relative time points in the CUP1 expression cycle.

We have performed ChIPs to measure the dynamics of acetylation of histones H3 and H4 in the strains shown in Fig. 2A, both to confirm Spt10-dependent acetylation in these strains, and to determine the dynamics of histone acetylation during CUP1 induction. The results of three independent ChIP experiments are shown in Fig. 2B, expressed as the level of immunoprecipitated material normalized to the input levels. These results show that Spt10-dependent H3 and H4 acetylation does occur at CUP1 in these strains. However, the kinetics of H3 and H4 acetylation are quite different in the wild-type strain. Acetylation of H3 (H3ac9/14) occurs within 10 min of copper addition, coinciding with maximum CUP1 expression, and decreases coincident with CUP1 down-regulation (Fig. 2B, top panel). The extent of H3 acetylation at 10 min is consistent with that observed at a similar time point (15 min) by Shen et al. (21). In contrast, H4 acetylation (measured at all sites, H4hyper) shows a decrease 10 min after copper addition, and a striking increase at 30 min after copper addition, when CUP1 transcription is down-regulated (Fig. 2B, bottom panel). The decrease in H4 acetylation after 10 min resembles that observed at 15 min by Deckert and Struhl (27). These results suggest that some of the discrepancies seen in previous studies could be explained by the dynamic changes in acetylation during CUP1 expression.

These results also indicate that opposing acetylation and deacetylation may be involved in both the activation and subsequent down-regulation of CUP1. If Spt10 is responsible for acetylation at both stages of CUP1 regulation, this protein may be in the unique position of mediating both induction and autoregulation of a single gene. Spt10 has been implicated in both gene activation and repression (25, 28–30), but never for the same gene. We therefore decided to further explore the role of Spt10 in CUP1 regulation.

spt10Δ-hta1 Double Mutants Alter CUP1 Expression Kinetics—The results shown in Figs. 1 and 2 indicate that both H2A and Spt10 are involved in the normal induction of CUP1. We decided to examine the effect of combining H2A mutations with spt10 deletion. We selected a number of strains containing H2A mutants in either the C or N terminus, and deleted the SPT10
gene in these strains. All of the double mutants were viable, though certain combinations (spt10Δ combined with htaS2A, htaS122A, or htaS129A) exhibit slow growth phenotypes; in these cases each of the single mutants shows moderately slow growth compared with wild type, which appears to be additive when these mutations are combined.

Combining spt10Δ and H2A mutations results in surprising changes in the patterns of CUP1 expression (Fig. 3). There were changes in the maximum levels of CUP1 mRNA, and in the autoregulation of CUP1 transcription. The most striking trend is that in every case, CUP1 transcription fails to shut down at 30 min after copper addition. In several instances, CUP1 mRNA levels are still increasing at 30 min.

It has been proposed that the shutdown of CUP1 is due to the binding of copper by the Cup1p metallothionein, which results in the release of Ace1p from the CUP1 UAS (31). This mechanism of autoregulation predicts that CUP1 shutoff simply requires that a sufficient level of Cup1p be synthesized to compete with Ace1p for copper binding. However, several of the mutants shown in Fig. 3 clearly accumulate large amounts of CUP1 mRNA without shutting down CUP1 expression. This suggests that CUP1 autoregulation may instead be a more active process dependent on Spt10 and/or H2A.

To explore this possibility, we performed more extensive analyses of the kinetics of CUP1 expression in the spt10Δ-hta1 double mutant strains. We took samples for Northern analysis every 10 min after copper addition, for 1–2 h. The results of 1 h time course are shown in Fig. 4A. Representative blots are shown on the left, and the averages of at least three independent experiments are presented in the graphs on the right.

The results from the long time course confirmed that CUP1 regulation is significantly altered in these strains, and further revealed different patterns of expression in different mutants. Fig. 4A shows that significant (though not complete) CUP1 shutoff occurs with only a slight delay in the spt10Δ strain, despite the low levels of CUP1 mRNA actually produced. This is consistent with the severe sensitivity to copper exhibited by spt10Δ cells (21), in that these cells do not appear to synthesize sufficient CUP1 mRNA for protection from the excess copper.

When the spt10 deletion is combined with mutations in H2A, the delay in induction observed in spt10Δ cells is preserved; however, most of these double mutants produce wild-type levels of CUP1 mRNA. Fig. 4B shows the results for spt10Δ-htaK5A and spt10Δ-htaK127A, which combine the spt10 deletion with a lysine-to-alanine mutation in each tail of H2A. Both of these strains show a peak of CUP1 expression at 20 min, coincident with the peak of expression in the spt10Δ mutant alone. However, these strains produce wild type or nearly wild-type levels of CUP1 mRNA, and exhibit a normal pattern of autoregulation. Spt10 (presumably via Spt10-dependent histone acetylation) is required for rapid induction of CUP1 within 5–10 min of copper exposure, and this rapid response is still absent in the double mutants. However, elimination of a positive charge on either H2A tail appears to alleviate the defect in the level of CUP1 induction in spt10Δ, possibly by mimicking an acetylation event that can partially substitute for an acetylation event that has been lost.

In contrast, the spt10Δ-htaT126Δ double mutant exhibits kinetics indistinguishable from spt10Δ alone (Fig. 4C). The nearby htaS129A mutation combined with spt10Δ, however, exhibits delayed (and incomplete) CUP1 shutoff, despite the accumulation of levels of CUP1 mRNA comparable to wild type.

The most striking misregulation of CUP1 occurs in spt10Δ-htaS2A and spt10Δ-htaS122A strains, shown in Fig. 4D. These strains show delayed induction (peaking at 20–30 min), but achieve wild-type levels of induction. What is surprising about these mutants is that they completely fail to autoregulate CUP1, but rather continue to express CUP1 at close to peak levels. This level of expression continues for at least 2 h (data not shown and Fig. 5, below).

These results strongly suggest that an active mechanism for CUP1 shutoff has been lost in these mutants. This regulatory mechanism is presumably not dependent on Ace1p displacement due to copper binding by Cup1p. The accumulation of CUP1 mRNA in these mutants far exceeds that of wild-type cells at the time of CUP1 shutoff in wild type; presumably a concomitant accumulation of Cup1p metallothionein also occurs. It is formally possible that translation of CUP1 mRNA is impaired in these mutants, though there is no general translational defect apparent in these mutants (with or without
copper) when total proteins are run on SDS-PAGE (data not shown). Cup1p protein itself is known to be quite stable, persisting well after CUP1 transcription shutdown (24).

What might be the mechanism of this active shutoff? Several possibilities present themselves. A specific repressor could bind to CUP1 and displace RNA polymerase or other positive factors (besides Ace1p) required for sustained transcription. Deacetylation of Spt10-acetylated histones H3 and H4 could be required to reset the “off” state for CUP1. A chromatin remodeler could act to return the nucleosomes at CUP1 to the positions normally occupied in the uninduced state. Whatever the mechanism, it is clear that this shutoff can be mediated by Spt10 or by serines in the H2A tails; loss of either alone does not impair shutoff, but the double mutants can. Any of the activities proposed could, for example, be recruited by Spt10p and stabilized at CUP1 by binding to (phosphorylated?) serines in H2A.

**swi1 Δ straın reveals kinetics reminiscent of a combination of both the spt10Δ strain and the spt10Δ-htaS2A, spt10Δ-htaS122A strains (Fig. 5A; compare with Fig. 4). swi1 Δ cells exhibit delayed CUP1 induction and reduced overall levels of CUP1 mRNA, similar to that**

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**FIG. 3. Deletion of SPT10 leads to altered CUP1 expression kinetics in strains bearing H2A point mutants.** Each panel shows the comparison of CUP1 expression in H2A point mutants alone to H2A point mutants combined with spt10 deletions. Representative Northern blots are shown below each graph. Quantitation and normalization of average data are as described in the legend to Fig. 1. All graphs have the same scale as the graph shown in Fig. 2A for ease of comparison.
seen for spt10Δ cells. However, as is the case for spt10Δ-htaS2A and spt10Δ-htaS122A strains, the swi1Δ cells also fail to shut off CUP1 transcription. This suggests two roles for SWI/SNF in CUP1 regulation. First, SWI/SNF may be involved in the initial Ace1p-dependent repositioning of nucleosomes that occurs during CUP1 induction (19). This repositioning is independent of Spt10-dependent histone acetylation (21); therefore, Spt10-dependent acetylation and nucleosome repositioning may be par-
tially redundant mechanisms facilitating access to the 
CUP1 promoter. Loss of either mechanism delays induction and re-
duces transcription efficiency; we predict that loss of both
mechanisms might result in failure to induce 
CUP1. Experi-
ments are currently underway to test this prediction.
The results shown in Fig. 5A also suggest a second role for
SWI/SNF, in 
CUP1 shutoff. While 
CUP1 mRNA levels in the
swi1Δ strain never achieve wild-type levels, the ongoing tran-
scription eventually leads to combined levels of 
CUP1 mRNA in excess of wild type. This is even more apparent in 2-h time
courses; examples of which are shown in Fig. 5B. After ~90 min
of copper exposure, wild-type cells begin to reactivate 
CUP1, though at least half the level of the initial burst of 
CUP1 expression (Fig. 5B, top panel). This is similar to previous
observations of the 
CUP1 response to 1 mM copper ion (24). In contrast, the swi1Δ (Fig. 5B, middle panel) and spt10Δ-htaS2A (Fig. 5B, bottom panel) strains maintain steady-state 
CUP1 expression at close to the maximum level achieved by the
respective strains. One model to explain these results is that
Spt10 is required to recruit SWI/SNF to 
CUP1, while interac-
tions with H2A are required to maintain SWI/SNF at
CUP1, or perhaps phosphorylation of H2A triggers the “resetting” activity
needed to turn off 
CUP1 expression.

**DISCUSSION**

We have shown that the normal kinetics of 
CUP1 induction and autoregulation depend on a complex interplay between
chromatin modifying enzymes and their histone targets. We have also shown that the shutdown of 
CUP1 may be a more active,
regulated process than was previously suspected. The fact that
different mutations in chromatin modifiers (spt10Δ + hta1 alleles versus swi1Δ) result in similar alterations in shutdown
kinetics argues that chromatin remodeling plays an active role
in turning off 
CUP1 shortly after its initial activation. Shut off
may normally involve the concerted action of several activities:
the acetylation of histone H4 by Spt10, the deacetylation of H3,
phosphorylation of H2A, and resetting of nucleosome positions
by SWI/SNF. Some of these effects could be indirect; for exam-
ple, SWI/SNF could be required for normal expression or re-
cruitment of a repressor needed for
CUP1 regulation. Detection
of SWI/SNF at 
CUP1 by ChIP, and measurement of nucleo-
some positioning during shut off, would support a direct role for
SWI/SNF remodeling.
The sensitivity of yeast cells to copper toxicity varies with
respect to cell cycle stage, with cells in G2/M being more sen-
sitive to killing by copper than cells in G1/S (32). This sensitivity
has been linked to variations in basal levels of Cu,Zn-
superoxide dismutase (Sod1) activity throughout the cell cycle
(33). An alternative explanation for the differences in 
CUP1 expression observed in the present study could be that different
mutants have different cell cycle rates or different distributions of
cell cycle stage occupancies, resulting in differential re-
sponses to copper. By monitoring budding, we do observe that
a larger percentage of logarithmically growing
spt10Δ-htaS2A cells occupy G2/M compared with wild type or spt10Δ alone
(~85% versus ~40–60%). Because this is the most copper-
sensitive stage of the cell cycle, this suggests that 1 mM copper
could be more stressful for this strain, perhaps triggering a
specific bypass of the normal 
CUP1 autoregulation process.

**FIG. 5.** swi1Δ deletion also alters CUP1 expression kinetics.
A, 60-min time course comparing wild type (squares) and swi1Δ CUP1 mRNA
levels. Representative Northern blots are shown on the left, average mRNA levels for 3 or more independent experiments are graphed on the right.
Quantitation and normalization of average data are as described in the legend to Fig. 1. B, 120-min time courses for wild type (squares), swi1Δ (diamonds), and spt10Δ-htaS2A (circles). The graph is a quantitation of the Northern blots on the left.
This “extra” stress could activate non-AceI-dependent pathways, such as the oxidative stress response, which could activate or maintain CUP1 expression in parallel with the AceI-dependent response. Future experiments will test whether alternate stress pathways are contributing to the response to copper in these strains. We do observe phosphorylation of histone H2A at Ser-129 in copper-treated cells, a modification normally associated with DNA double strand breaks (34). H2A phosphorylation has not been reported for other types of DNA damage; *Drosophila* H2Av, which is phosphorylated in response to double strand breaks, is not phosphorylated in response to oxidative stress (35). It will be important to test whether yeast H2A is phosphorylated at Ser-129 (or Ser-2, Ser-122) in response to oxidative stress per se.

Numerous studies have addressed the roles of histone modification and chromatin remodeling in gene silencing or repression, particularly in epigenetic silencing (for some recent reviews, see Refs. 8 and 36–39). However, there have been very few studies of the role of chromatin modifications in the process of shutting down an active gene. For many genes it is clear that shutdown is rapid and regulated, such as for genes transiently expressed prior to this study. For many genes it is clear that shutdown is rapid and regulated, such as for genes transiently expressed during the cell cycle, or genes that are autoregulated by their own products (such as CUP1). For genes for which rapid shutdown is important, it is likely that removal of specific activator(s) is one only part of the shutdown process. Besides their roles in repression or silencing, histone deacetylases have been implicated in transcription shutoff/chromatin resetting in some cases (22, 40, 41). One of these studies also implicated SWI/SNF in feedback repression (40). However, a dependence of normal shutdown kinetics on chromatin has not been addressed prior to this study.

One question that arises from this study is what is the role of rapid CUP1 shutoff in normal copper metabolism? High levels of Cup1 are known to protect cells in high copper conditions; regulated shut off of CUP1 could have serious repercussions for copper homeostasis. Cells may have evolved a rapid response to high copper levels, followed by a shut down for “reassessment.” If high copper levels persist, Ace1 is reactivated and appropriate levels of CUP1 transcription can resume. If the initial burst of Cup1 production was sufficient to bind the free copper, then it would be advantageous to not make excess Cup1, which could begin to compete with copper-dependent enzymes for their essential cofactor. This model leads to two possible testable outcomes for the mutants that do not shut down CUP1 appropriately: 1) that excess copper ion depletion might lead to induction of genes involved in copper uptake (i.e. simulation of a copper-starved state), and 2) that these strains might show decreased viability over time, as essential copper reserves are depleted.

This research emphasizes the complexity of copper homeostasis. Defects in copper homeostasis are responsible for serious disease in humans, including diseases of copper overload (Wilson’s disease), copper deficiency (Menkes disease), and carcinogenesis (42, 43). Detailed understanding of the regulation of the genes responsible for copper homeostasis provides not only insight into the complex interactions of chromatin remodeling in transcriptional control, but also provides critical understanding of processes essential to human health.

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Histone H2A and Spt10 Cooperate to Regulate Induction and Autoregulation of the *CUP1* Metallothionein
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