

Cti6 Is an Rpd3-Sin3 Histone Deacetylase-associated Protein Required for Growth under Iron-limiting Conditions in *Saccharomyces cerevisiae**[§]

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Iron and copper are redox active metals essential for life. In the budding yeast *Saccharomyces cerevisiae*, expression of iron and copper genes involved in metal acquisition and utilization is tightly regulated at the transcriptional level. In addition iron and copper metabolism are inextricably linked because of the dependence on copper as a co-factor for iron uptake or mobilization. To further identify genes that function in iron and copper homeostasis, we screened for novel yeast mutants defective for iron limiting growth and thereby identified the *CTI6* gene. Cti6 is a PHD finger-containing protein that has been shown to participate in the interaction of the Ssn6-Tup1 co-repressor with the Gcn5-containing SAGA chromatin-remodeling complex. In this report we show that *CTI6* mRNA levels are increased under iron-limiting conditions, and that *cti6* mutants display a growth defect under conditions of iron deprivation. Furthermore, we demonstrate that Cti6 is a nuclear protein that functionally associates with the Rpd3-Sin3 histone deacetylase complex involved in transcriptional repression. Cti6 demonstrates Rpd3-dependent transcriptional repression, and *cti6* mutants exhibit an enhanced silencing of telomeric, rDNA and *HMR* loci, similar to mutants in genes encoding other Rpd3-Sin3-associated proteins. Microarray experiments with *cti6* mutants grown under iron-limiting conditions show a down-regulation of telomeric genes and an up-regulation of Aft1 and Tup1 target genes involved in iron and oxygen regulation. Taken together, these data suggest a specific role for Cti6 in the regulation of gene expression under conditions of iron limitation.

Iron and copper are redox active metals that are essential for life in virtually all organisms and serve as catalytic co-factors for a wide variety of key cellular enzymes. Furthermore, much experimental evidence has established biochemical links between the ability of organisms to acquire copper and their ability to import iron into cells or distribute iron within cells or to peripheral tissues. Recent work on a high affinity iron uptake system in *Saccharomyces cerevisiae*, and on the multicop-

per oxidase ceruloplasmin in mammalian cells, firmly established the mechanistic requirement for copper as a redox co-factor that is essential for iron mobilization (1, 2). In Baker's yeast the Fet3 multicopper ferroxidase functions together with an iron permease (Ftr1) and ferric reductases (Fre1 and Fre2) at the plasma membrane in high affinity iron uptake. This reductive system of iron uptake is fully dependent on copper, a cofactor for the Fet3 multicopper oxidase (2). The ability of yeast cells to incorporate copper into Fet3 within a late secretory compartment requires copper uptake by the Ctr1 high affinity copper transporter and delivery of the copper by a cytosolic metallochaperone Atx1 to the copper-transporting ATPase Ccc2, localized in the trans Golgi network membrane. Ccc2 delivers copper into the lumen of the secretory pathway where it is loaded into Fet3 (for review see Ref. 3). Yeast mutants defective for copper uptake through *CTR1*, or delivery to the lumen of the secretory compartment via mutations in *ATX1* or *CCC2*, are unable to incorporate copper into the active sites of Fet3, resulting in defective high affinity iron uptake and a failure to grow under iron-limiting conditions. Consistent with this mechanism, the growth defect in limited iron and the absence of Fet3-dependent multicopper ferroxidase activity in *ctr1*, *atx1*, or *ccc2* mutants can be reversed by the addition of copper to the growth medium.

A key aspect to iron and copper homeostasis in *S. cerevisiae* is the regulation of transcription of genes encoding iron or copper homeostasis proteins in response to fluctuations in the availability of these two metals. Under conditions of copper limitation, the Mac1 copper-metalloregulatory transcription factor activates the expression of genes encoding components of the copper acquisition machinery that include the *CTR1* and *CTR3* high affinity plasma membrane copper transporters, the *FRE1* and *FRE7* metalloreductases, and other genes with as yet undescribed roles in copper homeostasis (2, 4–6). Under conditions of copper adequacy, copper acquisition genes are not expressed. In response to iron deprivation *S. cerevisiae* cells use two iron-responsive transcription factors, Aft1 and Aft2, to stimulate the expression of genes involved in iron acquisition, the so-called iron regulon (7). Targets for Aft1/Aft2 regulation include (i) genes which protein products are involved in high affinity reductive iron uptake such as the plasma membrane metalloreductases *FRE1–6* (8), the high affinity iron transport complex composed of the iron permease *FTR1* (9) and the multicopper oxidase *FET3* (10) and the *ATX1* copper chaperone and *CCC2* copper-transporting ATPase, (ii) genes encoding components of siderophore iron uptake systems, which include the transporters *ARN1–4* (11) and the cell wall manoproteins *FIT1–3* (12), (iii) genes involved in the mobilization of iron from vacuolar stores, which include the Fet3-Ftr1 homologue complex formed by *FET5* and *FTH1* (13) and the Nramp family

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[§] The on-line version of this article (available at <http://www.jbc.org>) contains Figs. S1–S3 and Tables S1–S4.

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member coded by *SMF3* (14, 15), (iv) the heme oxygenase homologue gene *HMX1* involved in regulation of intracellular heme levels (16), and other genes known or predicted to function in iron homeostasis are also transcriptionally activated by Aft1/Aft2 under iron deprivation. Therefore, copper and iron acquisition and distribution are regulated by metalloregulatory transcription factors that both activate or extinguish transcription in accordance with metal availability.

To explore additional aspects of the regulation of copper and iron acquisition, we screened a yeast haploid knock out library to identify genes that, when deleted, give rise to a growth defect under conditions of iron limitation that is rescued by exogenous iron and copper. One such mutant was identified in which the *CTI6* gene is insertionally deleted. Recent results show that Cti6 associates with the Ssn6(Cyc8)-Tup1 co-repressor (17). The Ssn6-Tup1 complex is recruited to target promoters by different DNA-binding repressors including Mig1, Crt1, Rox1, and Sko1, and mediates repression of genes specifically required for growth under adverse conditions such as glucose starvation, DNA damage, hypoxia, or osmotic stress, respectively (for a review, see Ref. 18). The mechanism of transcriptional repression includes, in addition to the interaction with the general transcription machinery, the specific interaction and recruitment of the Hda1, Rpd3, and Hos2 histone deacetylases (HDAC)¹ (19–23). Surprisingly, it has been shown that the Ssn6-Tup1 complex can also activate transcription of specific target promoters (*GAL1* and *ABN1*) by recruiting the Gcn5 HAT-containing SAGA complex (17, 24). This is possible because the Cti6 protein interacts simultaneously with the Ssn6-Tup1 and SAGA complexes, and mediates SAGA and TBP recruitment, histone acetylation, and transcriptional activation (17). Interestingly, recent studies in *S. cerevisiae* and *Schizosaccharomyces pombe* have also shown that the Tup1 complex (Tup11/Tup12 in *S. pombe*) modulates the expression of iron-regulated genes by associating with the Aft1 (Fep1 in *S. pombe*) transcription factor (25, 26).

Here we demonstrate that the *CTI6* gene is required for growth under iron-limiting conditions and for normal regulation of silencing. We demonstrate that *CTI6* is localized to the nucleus, associated with the Rpd3-Sin3 HDAC complex, and exhibits Rpd3 histone deacetylase-dependent transcriptional repression. Cti6 protein contains a PHD finger domain that is essential for growth under low iron and regulation of telomeric silencing, but not for the transcriptional repression activity. Finally, microarray experiments suggest that Cti6 may act as a repressor under low iron conditions in concert with the Ssn6-Tup1 corepressor. We discuss a potential role for Cti6, the Rpd3-Sin3 HDAC and the Ssn6-Tup1 complexes in cellular adaptive responses for growth under iron-limiting conditions.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—Genotypes for the yeast strains used in this work are listed in Supplemental Materials Table S1. To test growth on low iron, cells were grown in synthetic media (SC) to exponential phase ($A_{600} = 1.0$) and spotted in 10-fold serial dilutions starting at $A_{600} = 0.1$ onto SC alone (complete) or SC containing 75–100 μM BPS and 75–100 μM BCS (low iron/copper), or 1 mM ferrozine (not shown). For silencing assays, cells were spotted in 5-fold serial dilutions starting at $A_{600} = 1.0$ onto SC (complete), SC lacking specific requirements (SC minus), or SC containing 1 g/liter 5-fluoroorotic acid (FOA).

Plasmids—The wild type *CTI6* gene and C95A, H100A, and C95A,H100A mutant alleles were amplified by PCR with *Pfu* Turbo DNA polymerase (Stratagene), cloned into pRS416 (CEN, *URA3*) and pRS415 (CEN, *LEU2*) vectors using *SmaI* and *XhoI* restriction sites,

and sequenced. Wild type and mutant alleles of the *CTI6* gene were cloned in phase into pBTM116 (pADH-LexA) plasmid, a gift from Ann Vojtek (University of Michigan), using *SmaI* and *PstI* restriction sites. The *HDA1* gene was cloned into the pBTM116 plasmid using *SmaI* and *SalI* restriction sites. Site-directed mutagenesis of the *CTI6* gene was performed by the overlap extension method (27). M1835 (pADH-lexA) and M1836 (pADH-SIN3-LexA) plasmids were gifts from David Stillman (University of Utah), pJH330 (*INO1-LacZ*) from John Lopes (Wayne State University), and p6HA (*STE6-CYC1-LacZ*) from Ira Herskowitz (University of California, San Francisco).

DNA Microarray and RNA Blot Analysis—For microarray experiments wild type BY4741 and *cti6* mutant cells were grown to exponential phase in liquid SC medium containing 150 μM BPS and 150 μM BCS. Total RNA was isolated with a modified hot phenol method (28). RNA was further purified with a Qiagen RNeasy kit according to the manufacturer's instructions. The quality of the RNA samples was evaluated with an Agilent Bioanalyzer. Approximately 10 μg of total RNA from wild type and *cti6* cells was labeled with Cy3 and Cy5 fluorescent dyes, respectively. Cy-labeled RNA samples were hybridized with an Operon oligonucleotide yeast array. Data acquisition was performed using a GenePix Pro 4000A laser scanner (Axon Instruments). Only spots that had median values 2 times background were considered. For further information about preparation of the slides for microarrays, synthesis of fluorescent-labeled cDNA, hybridization, scanning and data acquisition, and quality control steps visit the Duke Microarray Core Facility web site. For RNA blot analysis, PCR-amplified fragments were radiolabeled with ³²P and used as probes.

β -Galactosidase Assays—Cells were grown in selective media to exponential phase. For *INO1-LacZ* assays 1 mM choline and 0.75 mM inositol were added to the medium. Cells were harvested and β -galactosidase activity was measured in permeabilized cells as previously described (29).

Fluorescence Microscopy—For Cti6 subcellular localization, wild type and mutant proteins were tagged with green fluorescent protein (GFP) at the carboxyl terminus as previously described (30). Cells were grown in selective media to exponential phase, and fluorescence visualized, photographed, and image processed as previously described (27). For staining of yeast nuclei cells were incubated for 15 min with 10 $\mu\text{g}/\text{ml}$ 4',6-diamidino-2-phenylindole.

RESULTS

***cti6* Mutants Exhibit a Growth Defect under Iron-limiting Conditions**—The acquisition of iron through the Fet3 multicopper ferroxidase is a copper-dependent process, and mutations in the copper uptake pathway (*ctr1*) or in the delivery of copper to the secretory compartment (*atx1* or *ccc2*) render cells defective in high affinity iron uptake, which can be corrected by increased exogenous copper levels. We screened a haploid yeast deletion library to ascertain if mutations in other loci conferred growth defects under iron-limited conditions that are rescued by extracellular iron and copper addition. One mutant, *cti6*, showed a dramatic growth defect under iron scarcity achieved by addition of the extracellular Fe(II) chelator BPS plus the Cu(I) chelator BCS (low iron/copper, Fig. 1, A and B). *cti6* growth defects were also observed by using the intracellular Fe(II) chelator ferrozine or BPS alone (data not shown), but because addition of BCS further decreases the availability of iron, BPS and BCS were used for further studies. Under these conditions *cti6* mutants show a growth defect similar to *ctr1*, *atx1*, and *ccc2* mutants (Fig. 1A). The growth defect of the *cti6* mutant was fully complemented by the addition of low concentrations of iron. Only 10 μM iron suppressed the growth defect of the *atx1* and *cti6* mutants, and up to 25 μM iron was required for growth recovery of *ctr1* and *ccc2* cells (Fig. 1A). Addition of copper also complemented the *cti6* growth defect (Fig. 1A). Addition of 5 μM copper stimulated growth of the *atx1* and *cti6* mutants. Higher copper concentrations robustly suppressed the growth defect of *cti6* and *ctr1* (25 μM), and *ccc2* (50 μM) mutants (Fig. 1A). *cti6* growth defect in low iron was also rescued by reintroducing the wild type or an allele in which the Cti6 carboxyl terminus was tagged with GFP in a centromeric plasmid under the control of *CTI6* wild type promoter (Fig. 1B). Taken together, these results demonstrate that *cti6* mutants

¹ The abbreviations used are: HDAC, histone deacetylase; BCS, bathocuproine disulfonic acid; BPS, bathophenanthroline disulfonic acid; FOA, 5-fluoroorotic acid; GFP, green fluorescent protein; PHD, plant homeodomain; TBP, TATA-binding protein; SC, synthetic media.

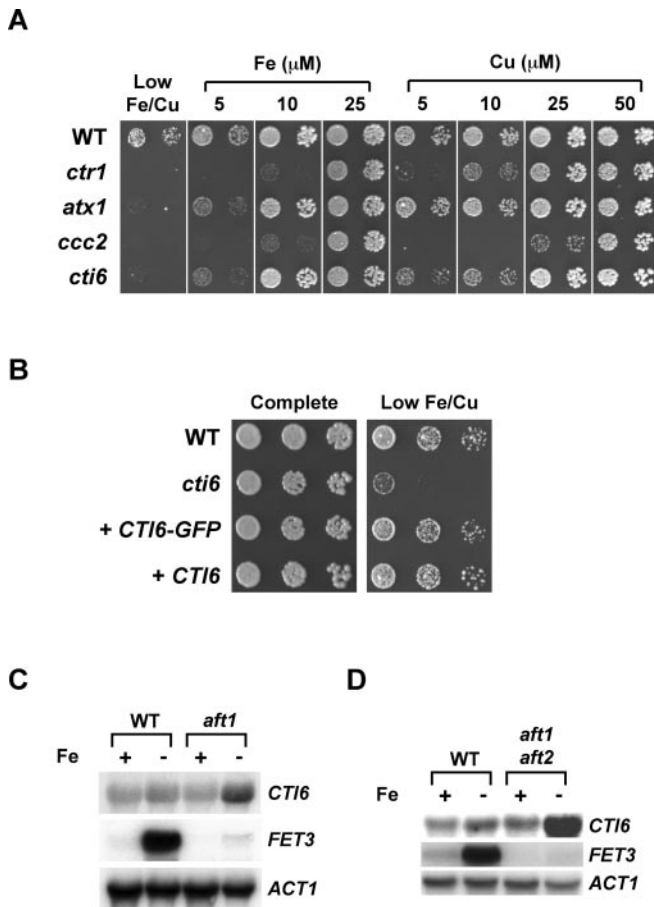


FIG. 1. A role for *CTI6* under iron-limiting conditions. A and B, *CTI6* is required for growth under iron-limiting conditions. 10-Fold serial dilutions starting at $A_{600} = 0.1$ of BY4741 wild type (WT), *ctr1*, *atx1*, *ccc2*, and *cti6* cells (A), and *cti6* transformed with pRS416 vector, pRS416-*CTI6*, and pRS416-*CTI6*-GFP (B) were assayed for growth on SC (complete) and SC containing 75 μM BPS and 75 μM BCS media (Low Fe/Cu) alone or with different concentrations of added iron ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$) or copper (CuSO_4). C and D, *CTI6* steady state mRNA levels are increased in iron-starved cells. BY4741 wild type (WT) and *aft1* cells were grown in low iron synthetic media containing 100 μM $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ (*Fe* +) or 100 μM BPS (*Fe* -) (C). CM3260 wild type and Y18aft2 (*aft1 aft2*) cells were grown in YPD containing 100 μM $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ (*Fe* +) or 100 μM BPS (*Fe* -) (D). Exponential cell growing cells ($\sim A_{600} = 1.0$) were collected, RNA extracted, and analyzed by RNA blotting with *CTI6*, *FET3*, and *ACT1* probes.

are defective for growth under conditions of iron scarcity and are rescued by addition of extracellular iron and copper.

***CTI6* mRNA Levels Are Increased under Low Iron Conditions**—Because the *CTI6* gene is required for growth under iron-limited conditions, we ascertained whether *CTI6* mRNA levels are regulated by iron availability. RNA blotting was performed using wild type cells, cells defective in the iron-sensing transcription factor Aft1 (*aft1*), and cells lacking both Aft1 and Aft2 (*aft1 aft2*). Cells were grown to exponential phase under iron-replete (+100 μM iron) and iron-limited (+100 μM BPS) conditions. The multicopper ferroxidase gene *FET3*, whose expression is induced under low iron conditions by the Aft1 transcription factor, was used as a control for iron-regulated gene expression. As previously reported (31), *FET3* mRNA levels increased under low iron conditions, and this induction was dependent on the Aft1 transcription factor (Fig. 1, C and D). However, the levels of *CTI6* mRNA were only slightly increased in wild type cells grown under iron limitation (Fig. 1, C and D). This *CTI6* basal expression was absent in a *cti6* deletion mutant (data not shown). *CTI6* mRNA levels under low iron conditions were considerably increased in both the *aft1* and *aft1 aft2* mutants, which sense a

much more severe iron starvation because of the loss of expression of genes involved in iron uptake (Fig. 1, C and D). Unlike *FET3*, the *CTI6* promoter lacks Aft1-Aft2-binding sites, consistent with our observation that Aft1 and Aft2 do not mediate the elevation in *CTI6* steady state mRNA levels in response to iron deprivation. Interestingly, increased *CTI6* (*YPL181w*) mRNA levels have also been reported previously in mutants defective in frataxin (Yfh1), a mitochondrial matrix protein required for iron-sulfur synthesis and export from mitochondria (32). Both *aft1* and *yfh1* mutant cells grown under low iron conditions show an increase in iron deficiency compared with wild type cells because of a defect in iron uptake and mobilization. Taken together, these results indicate that *CTI6* mRNA steady state levels increase during conditions of iron scarcity in a manner that is independent of the Aft1/2 iron-responsive transcription factors.

The *Cti6* PHD Finger Is Important for Growth under Iron-limiting Conditions—Cti6 is a PHD finger-containing protein. PHD fingers are zinc finger-like motifs of ~ 60 amino acids defined by seven cysteines and one histidine arranged as C_4HC_3 , with non-conserved intervening sequences. The PHD finger has been found in many eukaryotic proteins including transcription factors and proteins involved in chromatin-mediated transcriptional regulation (reviewed in Ref. 33). The structure of the PHD finger of the KAP-1 corepressor has been solved recently showing that it binds two zinc atoms in a cross-brace RING-like arrangement (34). According to this model, Cti6 protein could coordinate one zinc atom to cysteine residues 75, 77, and 103, and histidine residue 100 (site I), whereas the second zinc atom would coordinate to cysteine residues 92, 95, 117, and 120 (site II). To ascertain whether the PHD finger in the Cti6 protein is important for growth under conditions of iron deprivation, cysteine residue 95, a putative metal ligand for site II, and histidine residue 100, a predicted zinc ligand for site I, were converted to alanine by site-directed mutagenesis (Fig. 2A). Both single mutants were unable to complement the *cti6* growth defect under iron-limiting conditions (Fig. 2B). The double mutant C95A,H100A did not show any additional growth defect (Fig. 2B). Cti6 PHD finger mutants epitope-tagged with GFP were expressed at similar levels as wild type Cti6 and were properly localized (Fig. 3 and data not shown). These results strongly suggest that the integrity of the Cti6 PHD finger is important for growth under conditions of iron scarcity.

To ascertain the specificity of the iron limitation growth defect observed for *cti6* mutants we analyzed the growth rates, under iron-limited conditions, for the 15 additional mutants corresponding to genes in the *S. cerevisiae* genome coding for proteins containing PHD fingers. Of these, only cells harboring an insertional inactivation of the *PHO23* gene also showed a considerable growth defect under iron scarcity (Supplemental Materials Fig. S1). Interestingly, Pho23 has been recently shown to be a component of the Rpd3-Sin3 HDAC required for regulation of gene expression and silencing (35).

***Cti6* Is a Nuclear Protein**—The molecular function of the PHD finger motif has not been elucidated but, in analogy with LIM (C_2HC_5) and RING (C_3HC_4) domains, it has been suggested that PHD fingers could be involved in protein-protein interactions. Proteins containing PHD, LIM, or RING domains are often involved in transcriptional control either directly, or by recruiting co-repressors or co-activators (33). To obtain further insight into the function of Cti6 we epitope-tagged the protein at the carboxyl terminus with the GFP to determine its subcellular localization. Expression of the Cti6-GFP fusion protein under the control of the *CTI6* promoter fully rescued the growth defect observed for a *cti6* mutant strain under low iron conditions (Fig. 1C). Fluorescence microscopy of these cells

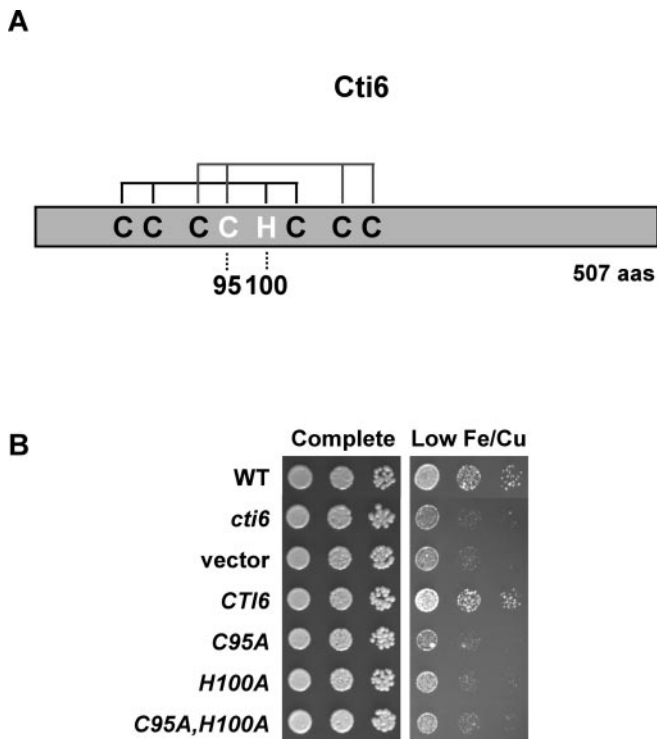


FIG. 2. The Cti6 PHD finger is required for growth under iron-limiting conditions. *A*, schematic representation of the PHD finger in Cti6 protein. Cysteine 95 and histidine 100 residues (*white characters*) where mutagenized to alanine. *B*, BY4741 wild type (WT), and *cti6* cells transformed with pRS416 (*vector*), pRS416-CTI6, pRS416-CTI6-C95A, pRS416-CTI6-H100A, and pRS416-CTI6-C95A,H100A were assayed for growth onto SC (complete) and SC containing 100 μ M BPS and 100 μ M BCS media (*Low Fe/Cu*).

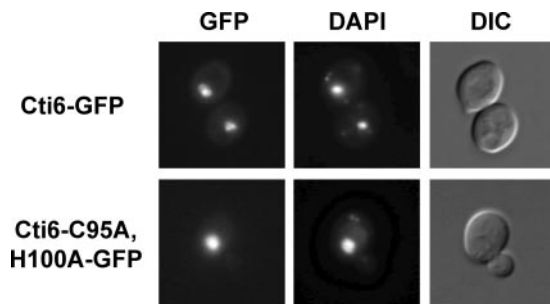


FIG. 3. Cti6 is a nuclear protein. *cti6* cells expressing pRS416-Cti6-GFP or pRS416-Cti6-C95A,H100A-GFP were grown in SC-ura to exponential phase, stained with 4',6-diamidino-2-phenylindole (DAPI), and visualized by fluorescence microscopy. The same nuclear localization for both wild type and mutant was observed when cells were grown in 100 μ M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, 100 μ M CuSO_4 , 100 μ M BPS or 100 μ M BCS containing media (data not shown). DIC, differential interference microscopy.

showed a Cti6-GFP fluorescence signal that overlapped with the nuclear stain 4',6-diamidino-2-phenylindole (Fig. 3, *top panels*). Mutagenesis of amino acid residues 95 and 100 (C95A,H100A mutant, Fig. 3, *bottom panels*), or growth under different concentrations of iron or copper availability (not shown) did not affect Cti6-GFP nuclear localization or protein levels. These results demonstrate that Cti6 is a nuclear protein, and strongly suggest that the integrity of the PHD finger, whereas essential for its role in cell growth under conditions of iron deprivation, is not required for its targeting to the nucleus.

A LexA-Cti6 Fusion Protein Mediates Rpd3-dependent Transcriptional Repression—Two independent reports have described large scale approaches to analyze multiprotein complexes in *S. cerevisiae* via the use of tandem affinity purification or FLAG epitopes (36, 37) to determine the identity

of associated proteins by affinity purification followed by mass spectroscopy. Interestingly, Cti6 (Ypl181w) protein was independently identified when Rpd3 and Sin3 were affinity purified using the tandem affinity purification epitope (36), and both Rpd3 and Sin3 copurified with Cti6 when the Ume1-Flag transcription factor was immunoprecipitated with anti-FLAG antibody (37) (Supplemental Materials Table S2). Rpd3 is a class I HDAC conserved from yeast to mammals. HDACs often mediate transcription repression by decreasing the state of histone acetylation. The Rpd3 HDAC is recruited to specific promoters by transcription factors such as Ume6 to regulate gene expression. In yeast, the Rpd3 HDAC forms a large multiprotein complex (~2 MDa) (38) with several associated proteins including Sin3, Sds3, Sap30, and Pho23, all of which are conserved in mammals (35, 39–42). Recently, it has been shown that Rpd3 is also required to activate osmoreponsive and heat stress genes (43).

The independent copurification of Cti6 with Rpd3 and Sin3 proteins by two groups strongly supports the hypothesis that Cti6 could functionally associate *in vivo* with the Rpd3-Sin3 complex in *S. cerevisiae*. To test this hypothesis we expressed a LexA-Cti6 fusion protein and ascertained the consequences of its expression on a *CYCI-UAS-LexA-LacZ* reporter, an assay previously used by others to test putative Rpd3-Sin3-associated genes (38, 39, 44). β -Galactosidase activity driven from this reporter showed an 18-fold decrease in the strain expressing the LexA-Cti6 fusion as compared with expression of LexA alone (Fig. 4, WT). This repression was reduced to a 1.4-fold value when the *RPD3* gene was deleted (Fig. 4, *rdp3*). As a control, the -fold repression mediated by a fusion of the histone deacetylase *HDA1* gene to LexA did not change considerably between wild type (7.3-fold repression) and *rdp3* mutant cells (8.3-fold repression) (Supplemental Materials Fig. S2). These results demonstrate that Cti6 mediates transcriptional repression when recruited to a promoter, and that this repression depends on the Rpd3 HDAC. In addition, transcriptional repression increased to 28–30-fold in the LexA-Cti6 PHD finger mutants (C95A, H100A, or both) when expressed in wild type cells (Fig. 4, WT), but dramatically decreased to 1.0–1.8-fold values in a *rdp3* mutant (Fig. 4, *rdp3*). These results indicate that the PHD finger integrity is not essential for Cti6-mediated repression when targeted to a promoter. Furthermore, the repression is dependent on Rpd3, suggesting that PHD mutations known to perturb the function of this domain in other proteins do not disrupt the functional interaction with the Rpd3 HDAC *in vivo*.

The Integrity of the Rpd3-Sin3 HDAC Complex Is Important for Growth under Iron-limiting Conditions—We have shown here that, in addition to Cti6, the Rpd3-Sin3 associated protein Pho23, which is also a PHD finger-containing protein, is also important for growth under conditions of iron limitation (Supplemental Materials Fig. S1). To determine whether the Rpd3-Sin3 complex is also required for growth under iron limitation, we tested the growth of mutants in *RPD3*, *SIN3*, and two additional genes encoding proteins associated with the Rpd3-Sin3 complex, *SDS3* and *SAP30*. As shown in Fig. 5, all Rpd3-Sin3 HDAC mutants show growth defects in low iron medium. The *rdp3* and *sin3* mutants that constitute the core of the complex exhibit a more severe growth defect under iron-limited conditions (data not shown). Taken together, these results demonstrate that the integrity of the Rpd3-Sin3 HDAC complex is important for growth under iron limitation.

We have demonstrated that *CTI6* mRNA levels increase under iron limitation conditions. In addition to Cti6, we have shown that other proteins associated with the Rpd3-Sin3 HDAC complex are also important for growth under iron scar-

FIG. 4. LexA-Cti6 transcriptional repression is dependent on *RPD3*.

The yeast strains SY641 (WT) and SY717 (*cti6*), containing the CYC1-UAS-LexA-LacZ reporter and transformed with pBTM116 (LexA), pLexA-Cti6 (*CTI6*), pLexA-Cti6-C95A (*C95A*), pLexA-Cti6-H100A (*H100A*), or pLexA-Cti6-C95A, H100A (*C95A,H100A*) plasmids, were grown on selective medium, and permeabilized cells were assayed for β -galactosidase activity (Miller units). The average of four independent transformants is represented with the S.D. -Fold repression was obtained by dividing the value obtained for cells expressing LexA with the value for cells containing LexA-Cti6 wild type or the mutant alleles. The same scale was used for both graphs.

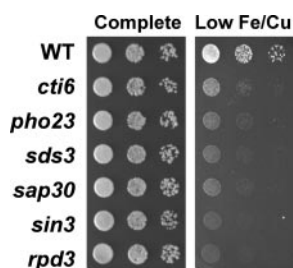
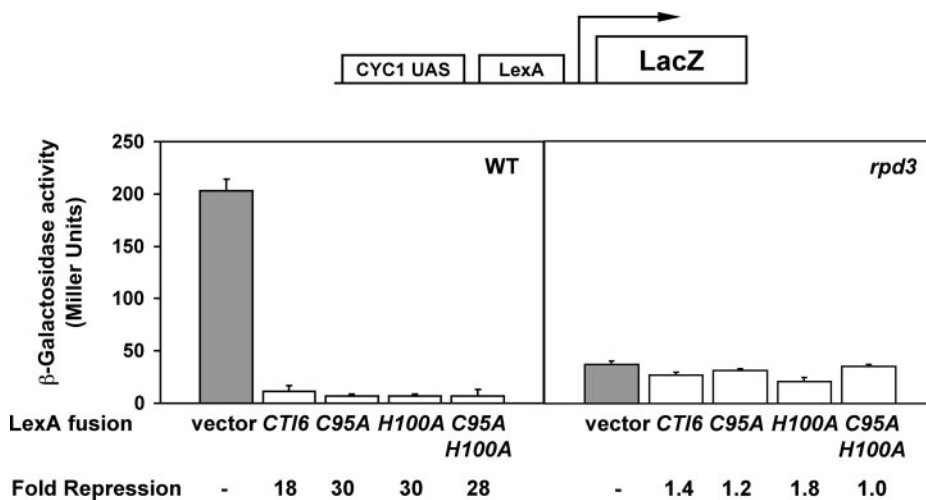


FIG. 5. The integrity of the Rpd3-Sin3 complex is required for growth under iron-limiting conditions. BY4741 wild type (WT), and *cti6*, *pho23*, *sds3*, *sin3*, and *rpd3* were assayed for growth under low iron/copper as described in the legend to Fig. 1B.

city. But does iron scarcity also increase the mRNA levels of these genes? No other member of the Rpd3-Sin3 HDAC complex analyzed (*RPD3*, *SIN3*, *PHO23*, *SAP30*, and *SDS3*) showed an increase in mRNA levels under low iron conditions (data not shown).

Cti6 Is Not an Essential Component of the Rpd3-Sin3 HDAC—Our data and published reports (36, 37) suggest that Cti6 functionally associates with the Rpd3-Sin3 HDAC, but do not ascertain whether Cti6 is an essential or a dispensable subunit of the complex. To evaluate the role of Cti6 in the Rpd3-Sin3 complex, we studied the repression of a *CYC1-LexA-LacZ* reporter mediated by a LexA-Sin3 fusion protein when compared with LexA alone. β -Galactosidase activity measurements demonstrate that wild type cells expressing LexA-Sin3 show a 20-fold repression of this reporter gene (Fig. 6). This repression was decreased to 4.5-fold in an *rpd3* strain, but remained unaffected in the *cti6* mutant (Fig. 6). Therefore, Cti6 is not required for the transcriptional repression mediated by Sin3 of this reporter gene.

To investigate how physiological targets for the Rpd3-Sin3 HDAC complex, such as *INO1* and *IME2*, were expressed in a *cti6* mutant, we used strains harboring *INO1-LacZ*, *IME2-LacZ*, and *STE6-LacZ* reporter constructs. Both *rpd3* and *sin3* mutant strains showed a 20–30-fold increase in β -galactosidase activity for *INO2* and *IME2* as previously reported (39), whereas their expression did not change in *cti6* mutants compared with wild type cells (Supplemental Materials Fig. S3). β -Galactosidase activity of *STE6* was 4.5-fold decreased in *rpd3* and *sin3* strains compared with wild type, as previously shown (39). Interestingly, *cti6* mutants showed only a 2-fold decrease in *STE6* compared with wild type cells (Supplemental Materials Fig. S3). Therefore, whereas Cti6 does not affect some Rpd3-Sin3-regulated genes such as *INO1* and *IME2*, other Rpd3-Sin3 targets, such as *STE6*, are indeed misregu-

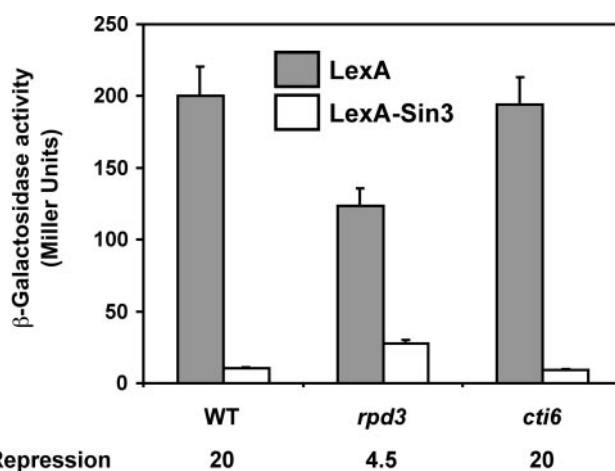


FIG. 6. *CTI6* is not required for LexA-Sin3 transcriptional repression. The yeast strains SY641 (WT), SY717 (*rpd3*), and SPY115 (*cti6*), transformed with either M1835 (LexA) or M1836 (LexA-Sin3) plasmids, were grown on selective medium, and permeabilized cells were assayed for β -galactosidase activity. -Fold repression was obtained by dividing the value obtained for cells expressing LexA with the value for cells containing LexA-Sin3. The average of four independent transformants is represented with the S.D.

lated in a *cti6* mutant. Taken together, therefore, these results leave open the possibility of Cti6 participating as an accessory and regulatory subunit or by recruiting the HDAC complex to specific target sites in the genome.

cti6 Mutants Exhibit an Enhanced Silencing at Telomeric, rDNA and HMR Loci—Despite the battery of observations demonstrating that the Rpd3-Sin3 HDAC complex is a transcriptional repressor, the transcription profile of the *rpd3* and *sin3* mutants grown in rich media documents more genes being down-regulated (~265) than up-regulated (~170) (45, 46). Most of the genes whose expression is reduced in cells defective in members of the Rpd3-Sin3 HDAC complex are located in the telomeric and subtelomeric regions of the chromosomes (45). The mechanisms underlying this repression are currently unknown but, because chromatin immunoprecipitation experiments have shown that Rpd3 is excluded from the telomeric and subtelomeric domains (22, 47), it has been proposed that the up-regulation of histone genes in *rpd3* mutants could be responsible for this enhanced silencing (45). Therefore, the repression at these, and at other silenced loci in yeast including rDNA and mating-type loci, may be a consequence of an indirect mechanism. In addition, it has been previously reported that mutants in the *RPD3* or *SIN3* genes show a more severe

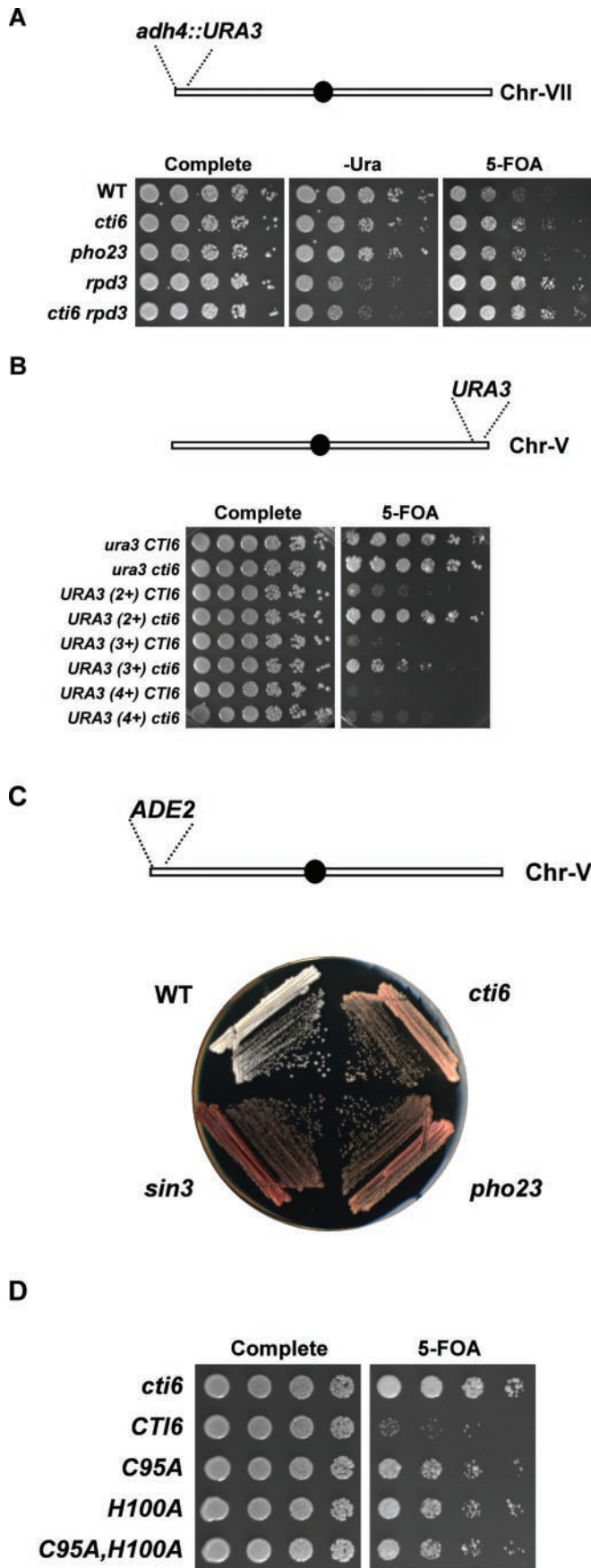


FIG. 7. *cti6* mutants exhibit an enhanced telomeric silencing. A, telomeric silencing was assayed by using a strain containing a *URA3* reporter gene integrated at the *adh4* locus located at 15 kb from the left-end of chromosome VII. 5-Fold serial dilutions starting at $A_{600} = 1.0$ were spotted onto SC (complete), SC-ura (*-Ura*), and 5-FOA plates.

silencing phenotype than mutants defective in other genes associated with the complex such as *SAP30* and *PHO23* (35). We made use of multiple approaches to ascertain the effect of *cti6* mutants in gene expression at the different silenced loci in *S. cerevisiae*: telomeres, ribosomal DNA, and mating-type loci.

We first assayed telomere silencing using a strain with the sole *URA3* gene integrated into the *adh4* locus (*adh4::URA3*), which is located at 15 kb from the left-end of chromosome VII (Fig. 7A). It has been previously reported that *rdp3*, *sin3*, and *sds3* mutants show strong silencing at this locus (39), whereas *pho23* mutants exhibit a milder silencing phenotype (5-fold difference from wild type) (35). We deleted *CTI6* in this strain background and compared cell growth in the absence of uracil (*-Ura*) or in the presence of 5-FOA to wild type cells and to *rdp3* or *pho23* mutants by 5-fold serial dilutions. As compared with wild type cells, *rdp3* mutants showed a growth defect in minus uracil, which is consistent with enhanced silencing at the *adh4::URA3* locus (Fig. 7A, *-Ura*). On the contrary, *cti6* and *pho23* mutants only showed a minor growth defect under these conditions (Fig. 7A, *-Ura*). Silencing was more clearly observed in *cti6* and *pho23* mutants when grown in the presence of the Ura^+ toxic compound 5-FOA. Wild type cells showed a more severe growth defect in 5-FOA compared with *cti6*, *pho23*, and *rdp3* strains, which is indicative of a higher *URA3* expression. Importantly, the telomeric silencing phenotype for a *cti6 rpd3* double mutant was indistinguishable from the *rdp3* single mutant (Fig. 7A, *-Ura*). This lack of a *cti6* additive effect on the telomeric silencing shown by *rdp3* mutants suggests that both proteins function in the same genetic pathway.

To further examine the telomeric silencing phenotype of the *cti6* mutant, we evaluated silencing at the right telomere of chromosome V by two different strategies. We first used a set of strains containing the *URA3* reporter inserted at 2.0, 3.5, and 6.5 kb from the end of the chromosome (2+, 3+, and 4+ strains, respectively). Deletion of *CTI6* strongly enhanced growth in 5-FOA for strains 2+ and 3+, and modestly enhanced growth in the 4+ strain, which is consistent with a strong Cti6-dependent silencing of the *URA3* gene located in the telomere (Fig. 7B). Second, we used a strain containing a telomeric *ADE2* reporter (Fig. 7C). The pink colony color developed by *ade2* mutants was used as a qualitative assay for telomeric silencing. Whereas colonies from the isogenic wild type strain were white, *cti6*, *pho23*, and *sin3* mutants showed qualitative increases in pink colony color, respectively (Fig. 7C), which is indicative of a strengthening in telomeric silencing. Taken together, these results strongly suggest that Cti6 modulates telomeric silencing in a manner similar to other members of the Rpd3-Sin3 HDAC complex.

The strains used were DY5888 (wild type; *WT*), SPY117 (*cti6*), SPY168 (*pho23*), DY5894 (*rdp3*), and SPY188 (*cti6 rpd3*). Less growth on *-Ura* and increased growth on 5-FOA plates are indicative of silencing. B, telomeric silencing was assayed by using a strain containing a *URA3* gene integrated at 2.0 (2+), 3.5 (3+), and 6.5 (4+) kb from the right-end of chromosome V. 5-Fold serial dilutions were spotted onto SC (complete) and 5-FOA plates. The strains used were YPH250 (*ura3 CTI6*), SPY181 (*ura3 cti6*), UCC506 (*URA3 (2+) CTI6*), SPY182 (*URA3 (2+) cti6*), UCC508 (*URA3 (3+) CTI6*), SPY183 (*URA3 (3+) cti6*), UCC510 (*URA3 (4+) CTI6*), and SPY184 (*URA3 (4+) cti6*). C, silencing at telomeres was assayed by using a strain containing a *ADE2* reporter gene located in the left telomere of chromosome V. Cells were grown on SC with limiting amounts of adenine. The strains used were YNB9 (*WT*), JS756 (*sin3*), JS758 (*pho23*), and SPY176 (*cti6*). Because *ade* minus cells are pink, an increased color is indicative of enhanced silencing. D, mutagenesis of the Cti6 PHD finger increases telomeric silencing. SPY182 (*URA3 (2+) cti6*) strain transformed with pRS415 vector (*cti6*), pRS415-*CTI6* (*CTI6*), pRS415-*CTI6*-C95A (*C95A*), pRS415-*CTI6*-H100A (*H100A*), and pRS415-*CTI6*-C95A,H100A (*C95A, H100A*) was grown on SC-Leu (complete) and 5-FOA plates as described in B.

We previously showed that Cti6 PHD finger integrity is important for cell growth under iron-limiting conditions (Fig. 2B) but not for Rpd3-Sin3-mediated repression of a reporter gene (Fig. 4). We asked whether telomeric silencing is also dependent on this domain. We transformed the *cti6* strain containing the *URA3* gene positioned 2 kb from the right-end of chromosome V (Fig. 7B, *URA3* (2+) *cti6* strain) with vector alone, wild type *CTI6*, and the PHD finger mutants C95A, H100A, and the C95A,H100A double mutant. Wild type *CTI6* dramatically decreased growth in 5-FOA plates, which is consistent with an abolishment of the enhanced silencing induced by the *cti6* mutant (Fig. 7D). Importantly, *CTI6* PHD finger mutants did not show the same severe growth defect in 5-FOA as the *CTI6* wild type, but rather a slight growth delay compared with the *cti6* mutant. These results suggest that whereas the integrity of the Cti6 PHD finger is not crucial for genetic interaction with the Rpd3 HDAC (Fig. 4), it is important for iron-limited growth and for the regulation of telomeric silencing.

Because *rdp3*, *sin3*, *sap30*, and *pho23* mutants enhance silencing at the ribosomal DNA and mating-type loci, we tested the effect of *CTI6* deletion. By using a strain containing a *URA3* and *HIS3* reporter at the rDNA locus, we observed a growth defect in minus uracil/histidine plates for *cti6* mutants compared with wild type (Fig. 8A). The growth defect observed for the *cti6* strain was similar to the *pho23* and *sap30* mutants, but less severe than that shown by the *rdp3* and *sin3* mutants. A colorimetric assay, using a strain containing a *rDNA::MET15* reporter, also supported increased ribosomal silencing in *cti6* mutants. This is indicated by the darker colony color shown by *cti6*, *pho23*, and *sin3* mutants compared with the wild type strain (Fig. 8B). Finally, an *hmrΔA::TRP1* reporter was used to test silencing at the mating locus. The result showed a growth defect for *cti6* mutant in minus tryptophan (Fig. 8C), which is consistent with enhanced silencing at this locus. In summary, these results strongly suggest that Cti6 modulates genomic silencing at the telomeric, rDNA, and mating-type loci, in a manner similar to other Rpd3-Sin3 HDAC components.

Genome-wide Analysis of Gene Expression in *cti6* Mutants under Low Iron Conditions Shows Up-regulation of Iron and Oxygen-related Genes—To provide further mechanistic insight on the function of Cti6 under low iron conditions, and its role in the Rpd3-Sin3 repressor complex, we performed a genome-wide microarray analysis. We compared gene expression in wild type and *cti6* mutant cells grown under low iron and copper conditions. Results from two independent experiments showed that at least 59 genes were down-regulated and 110 genes up-regulated in both microarrays by at least 1.4-fold (Supplemental Materials Tables S3 and S4). Interestingly, 40% (24 of 59) of the genes down-regulated in *cti6* cells were located in chromosomal telomeric regions. This result further emphasizes the function of Cti6 in telomeric silencing regulation. Eleven transposon elements and eight genes of unknown function were also repressed in the *cti6* mutant (Supplemental Materials Table S3). The remaining 16 down-regulated genes have diverse functions including pheromone regulation (*STE3*, *PRM7*, and *PRM10*), stress (*SSA3*, *SPG4*, *SNZ1*, and *SIP4*), and others (*FMT1*, *TKL2*, *STP4*, *PDC6*, *PDR11*, *RRP14*, *YSR3*, *MLP1*, and *BAG7*). Importantly, the majority of non-telomeric genes affected in both microarrays were genes up-regulated in the *cti6* mutant (76%: 110 of 145). This result further supports a repressive role of gene expression for the Cti6 protein. Genes increased in a *cti6* mutant compared with wild type cells under low iron conditions include genes involved in a wide variety of cellular functions such as iron and oxygen (11 genes), membrane transporters (10 genes), glucose (8 genes), secretory pathway (14 genes), cell wall and external medium (11 genes),

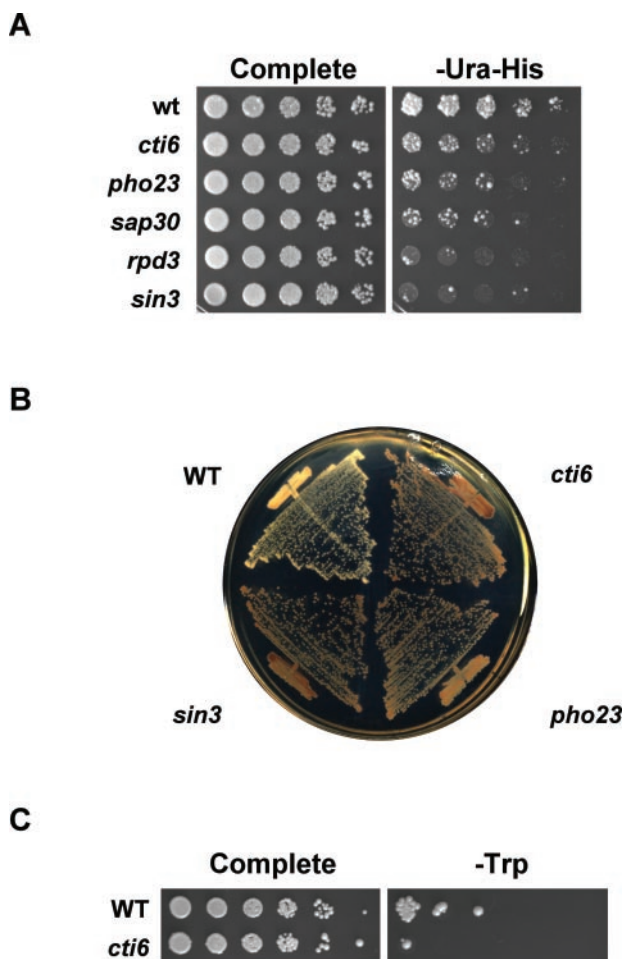


FIG. 8. *cti6* mutants exhibit enhanced silencing at rDNA and HMR loci. A, rDNA silencing was assayed by using a strain containing a *URA3/HIS3* reporter cassette integrated at the rDNA locus. 5-Fold serial dilutions starting at $A_{600} = 1.0$ were spotted onto SC (complete) and SC-ura-his ($-Ura - His$). Decreased growth on SC-ura-His is indicative of an enhanced silencing. The strains used were JS311 (wild type; WT), SPY175 (*cti6*), JS767 (*pho23*), M475 (*sap30*), M480 (*rdp3*), and JS493 (*sin3*). B, rDNA silencing was assayed by using a strain containing a *MET15* reporter cassette integrated at the rDNA locus. Cells were grown onto YPD plates containing 0.07% $Pb(NO_3)_2$. The strains used were YNB9 (WT), JS756 (*sin3*), JS758 (*pho23*), and SPY176 (*cti6*). Because *MET15* defective cells develop a dark color in the presence of $Pb(NO_3)_2$, a darker colony color is indicative of an increased silencing. C, silencing at the *HMR* locus was assayed by using a strain containing a *TRP1* reporter in a *HMRΔA* mutated locus. 5-Fold serial dilutions of JLS59 (WT) and SPY187 (*cti6*) strains were spotted onto SC (complete) and SC-trp ($-Trp$). Lack of growth on minus Trp is indicative of silencing.

pheromone regulation (4 genes) among others. For a complete list see Supplemental Materials Table S4.

Both microarray (Supplemental Materials Table S4) and Northern blot analysis (Fig. 9, and data not shown) showed that four direct Aft1/2 targets (*FIT2*, *FIT3*, *HMX1*, and *SMF3*) were up-regulated in a *cti6* mutant suggesting a misregulation of the iron homeostasis machinery. In addition, all the subunits of the ribonucleotide-diphosphate reductase (*RNR1-4*), including the subunit containing the di-iron tyrosyl radical, were increased in *cti6* mutants. The enzyme ribonucleotide reductase catalyzes the rate-limiting step in deoxyribonucleotide synthesis, playing an essential iron-dependent role in DNA replication and repair (48). Interestingly, expression of the *RNR2* and *RNR3* genes is repressed by the Ssn6-Tup1 complex. Furthermore, genes belonging to two families of aerobic repressed genes were increased in *cti6* mutants under low iron

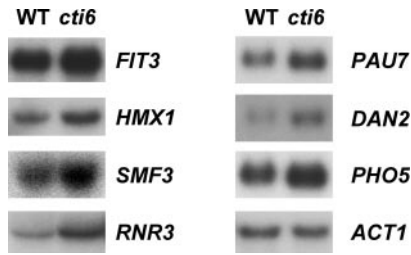


FIG. 9. **Increased expression of iron- and oxygen-related genes in a *cti6* mutant.** BY4741 wild type (*WT*) and *cti6* cells were grown in low iron/copper media (SC containing 150 μ M BPS and 150 μ M BCS) to exponential growth phase. RNA was extracted and analyzed by RNA blotting with *FIT3*, *HMX1*, *SMF3*, *RNR3*, *PAU7*, *DAN2*, *PHO5*, and *ACT1* probes.

conditions. These genes include members of the seripauperin family (*PAU1* and *PAU7*) (49) and the cell wall manoprotein family DAN/TIR (*DAN2*) (50). Repression of both PAU and DAN genes under aerobic conditions is mediated by heme in a mechanism involving the Ssn6-Tup1 repressor complex (49, 50). In conclusion, our genome-wide analysis of gene expression under limiting iron/copper conditions indicates that (i) genes down-regulated in a *cti6* mutant are mostly located in telomeres, (ii) Cti6 acts as a repressor affecting a wide variety of genes, and (iii) several iron- and oxygen-regulated genes are increased in a mechanism that could involve the Ssn6-Tup1 repressor complex.

DISCUSSION

To further understand the mechanisms for copper and iron homeostatic regulation in yeast, we performed a screening to identify genes required for growth under iron limitation conditions. One such gene identified was *CTI6* (*YPL181w*). Genome-wide protein-protein interaction studies have suggested that Cti6 associates with the Rpd3-Sin3 HDAC multiprotein complex in yeast (36, 37). To completely understand the function and regulation of the Rpd3 HDAC in cells, it is crucial to elucidate the role of proteins that function within this large complex under different environmental conditions. In this report, we provide evidence to support a functional association of Cti6 with the Rpd3-Sin3 HDAC complex that impacts growth under conditions of iron limitation. First, we show that Cti6 is able to repress transcription when tethered to a promoter, and this repression activity is dependent on the *RPD3* gene. Second, cells defective in *CTI6* show several phenotypes similar to mutants in subunits of the Rpd3-Sin3 HDAC complex, including an enhanced silencing at telomeric, rDNA and mating type loci. In addition, the *cti6 rpd3* double mutant does not enhance the telomeric silencing shown by the *rpd3* single mutant, further supporting the hypothesis that both proteins function in the same biochemical pathway. Additional data presented here also indicate that Cti6 is not an essential component of the Rpd3-Sin3 HDAC complex. First, Sin3-mediated repression, which is dependent on Rpd3, is completely independent of Cti6. Second, *INO1* and *IME2* expression, which is increased in *rpd3* and *sin3* mutants, is not affected in *cti6* cells. And third, other phenotypes associated with defects in the Rpd3-Sin3 HDAC complex, such as sensitivity to cycloheximide and heat shock, were not observed for *cti6* mutants.² Therefore, the data presented in this report strongly suggest that Cti6 associates with the Rpd3-Sin3 HDAC complex, and perhaps targets, or otherwise regulates the specificity or catalytic activity of this important regulatory complex under specific environmental conditions.

Recent reports show that the Ssn6-Tup1 co-repressor complex remains associated with some specific target promoters

(glucose starvation, hypoxic, and osmotic stress targets) under stress inducing conditions (17, 24). Cti6 protein plays a key role in this process by interacting with the Ssn6 repressor and the Gcn5 histone acetylase-containing complex SAGA, mediating SAGA and TBP recruitment, histone acetylation, and finally transcriptional activation of genes such as *GAL1* and *ANB1* (17). In addition, cell type-specific and DNA damage-inducible promoters are only occupied by the Ssn6-Tup1 complex under repressive conditions (51, 52) ruling out any involvement of Ssn6-Tup1 in activation of this set of genes. More recently, it has been shown that the recruitment of the Ssn6-Tup1 complex to iron-regulated promoters such as *FRE2* and *ARN2* is essential for gene activation (26). In addition, studies in *S. cerevisiae* and *Candida albicans* have shown that cells defective in Tup1 de-repress the iron-siderophore uptake system (53, 54). Furthermore, Tup1 homologues in *S. pombe* (Tup11 and Tup12) are required for transcriptional repression of components of the iron transport machinery through a mechanism involving direct interaction with the GATA transcription factor Fep1, which is involved in repression of iron transport in response to elevated iron concentrations (25, 55). Our genomic microarray and RNA blot analysis for wild type and *cti6* mutant cells under conditions of iron limitation shows that several Tup1 target genes including the RNR genes, involved in DNA damage and replication, and the PAU and DAN families, expressed under anaerobic conditions, are up-regulated in the *cti6* mutant, suggesting that Cti6 mediates transcriptional repression through the Ssn6-Tup1 corepressor complex. Taken together these observations lead to a hypothesis where the Ssn6-Tup1 complex may recruit the Rpd3-Sin3 HDAC complex via the interaction with Cti6 repressing or, as recently described (43), activating gene expression.

As expected for a gene involved in genome-wide transcriptional repression (and activation through its interaction with the Rpd3-Sin3 and Ssn6-Tup1 complexes), many genes located in telomeric regions or involved in functions as diverse as ribosomal genes, pheromone regulation, glucose metabolism, membrane transporters, cell wall, and vesicle trafficking are misregulated in a *cti6* mutant under iron limitation. The misregulation of genes coding for ribosomal proteins and pheromone-related gene products could be a consequence of the increased silencing that we have shown for the rDNA and HMR loci in *cti6* mutants. Recent genome-wide studies have identified 15 different gene mutations that confer synthetic lethality in combination with *cti6* mutation (56). Interestingly, seven out of them are involved in the incorporation of the H2A-histone variant H2A.Z (Htz1 in yeast) to chromatin, and include *HTZ1* itself and *SWR1*, the catalytic core of the chromatin remodeling complex (57, 58). Similarly to *cti6* mutants, *htz1* mutant cells show activation of genes located in telomeric and HMR loci (59). In addition, several members of the Swr1 complex also identified in this study such as *SWC3*, *ARP6*, *VPS71*, and *VPS72*, are involved in endoplasmic reticulum biogenesis and vacuolar protein sorting (60, 61). Other genes showing synthetic lethality with *CTI6* include *THI3*, involved in thiamine biosynthesis, the mitochondrial inner membrane translocase *TIM13*, the pheromone-related gene *PRM6*, transcription elongation factors *CDC73* and *LEO1*, the HDACs *HOS2* and *SIF2*, and actin-related gene *PAC10* (56). Despite the pleiotropic effects observed for *cti6* mutants, several lines of evidence presented in this report strongly suggest a role for Cti6 under iron limitation. First, *cti6* mutants exhibit a severe growth defect under low iron media. Second, *CTI6* mRNA steady-state levels increase under nutritional and genetic iron deficiency. And third, several Aft1/2 as well as Tup1-dependent genes are up-regulated in the *cti6* mutant grown under iron limitation conditions.

The Cti6 protein contains a C₄HC₃ motif conserved among

² S. Puig, M. Lau, and D. J. Thiele, unpublished observations.

eukaryotes called PHD finger, which is usually found in proteins involved in chromatin-mediated transcriptional regulation (33). Point mutations or deletion of PDH fingers contributes to a variety of human diseases including α -thalassemia and mental retardation syndrome, autoimmune dysfunction, and myeloid leukemias. Therefore the study of the precise function of PHD fingers, which is currently unknown, will be of importance in understanding the basis of many human diseases. Preliminary studies by Tzamarias and co-workers (17) suggested that the Cti6 PHD motif is not required for interaction with either Ssn6 or the SAGA complex, or binding to the GAL1 promoter, but is essential for gene activation at this locus. Similarly, we show that the integrity of the PHD finger motif in the Cti6 protein does not affect nuclear localization and Rpd3-mediated repression (LexA-Cti6 assays), which suggests that the PHD motifs are not essential for interaction with the Rpd3-Sin3 multiprotein complex, but they are required for growth under iron deprivation conditions and telomere silencing regulation. Because several members of the Rpd3-Sin3 HDAC complex are required for both, growth under iron limited conditions and silencing regulation, we hypothesize that the Cti6 PHD finger might be involved in protein-protein interactions with a specific member of the Rpd3-Sin3 HDAC complex required for this function. On the other hand, the Cti6 PHD finger could also be required for its recruitment to chromatin, which is bypassed by LexA tethering in our experiments.

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