The Tup1-Cyc8 Protein Complex Can Shift from a Transcriptional Co-repressor to a Transcriptional Co-activator*

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Cyc8(Ssn6)-Tup1, a general co-repressor complex, is recruited to promoter DNA via interactions with DNA-binding regulatory proteins and inhibits the transcription of many different yeast genes. Previous studies have established that repression function of the complex is performed by one subunit of the complex, the Tup1 protein, and requires specific components of the RNA polymerase II holoenzyme such as Sin4 and Rgr1. In this study we test the transcriptional activity of the Cyc8 subunit using a LexA operator-containing reporter. We show that a LexA-Cyc8 hybrid stimulates transcription when expressed in a tup1Δ, a sin4Δ, or a rgr1Δ strain, suggesting that transcriptional activation is an intrinsic property of the Cyc8-Tup1 co-repressor. In support of this notion we demonstrate that Cyc8-Tup1 has a dual function on CIT2, a gene encoding a citrate synthase that is expressed upon mitochondrial dysfunction. First, we show that Cyc8-Tup1 is tethered to CIT2 promoter by interacting with the activation domain of Rtg5, a bHLH/L-Zip DNA-binding transactivator of CIT2. Next we demonstrate that Cyc8-Tup1 activates CIT2 transcription in response to mitochondrial dysfunction, and this stimulatory effect is mediated by Cyc8. In contrast, basal (noninduced) expression of this gene is inhibited by Tup1. These findings establish a positive role for the Cyc8-Tup1 complex in transcription and support a model by which specific metabolic signals may convert the Cyc8-Tup1 transcriptional co-repressor to a co-activator of certain promoters.

An important class of pleiotropic transcriptional regulators includes intermediary proteins such as co-activators and co-repressors. These protein factors are tethered to specific promoters mainly by contacting DNA-binding factors and regulate transcription either by interacting with components of the Pol II holoenzyme or by modifying chromatin structure, or both (1, 2). The human co-activator CBP/p300 (3), the yeast SAGA complex (4), and the human nuclear receptor co-repressors SMRT and N-CoR (5) are among the best characterized examples of this growing protein family. Interestingly, some of these factors have dual function on specific promoters; for example CBP/p300, which mediates activation of interferon β gene expression in response to virus induction is also responsible for post-induction turn off (35).

In the yeast *Saccharomyces cerevisiae*, two physically associated proteins, Cyc8(Ssn6) and Tup1, inhibit the transcription of many diversely regulated genes when their expression is not required (6–8). It is well established that Cyc8-Tup1 acts as a co-repressor complex that does not bind DNA directly but is recruited to different promoters via interactions with specific DNA-binding regulatory proteins. The repression function of the complex is performed by a specific domain of Tup1 (8). When the Tup1 repression domain is brought upstream of an active test promoter through the DNA binding domain of LexA, it inhibits transcription independently of Cyc8. Moreover, this domain is required for repression of natural genes such as glucose, oxygen, and cell-type regulated genes. It has been postulated that multiple mechanisms are responsible for Tup1 repression. Tup1 interacts with histones H3 and H4 and may position nucleosomes over the transcription start point, suggesting that Tup1 might repress transcription by modifying chromatin structure (9, 10). However, evidence from other studies argue that Tup1 inhibits the function of the basic transcription machinery; Tup1 repression was reconstituted in an *in vitro* transcription system in the absence of chromatin (11), and mutations in specific components of the RNA polymerase II holoenzyme complex, such as the mediator proteins Sin4, Rgr1, Srb10, and Srb11, weaken the Tup1 repression activity (12–15).

Previous studies suggested that Cyc8 does not directly inhibit transcription but contacts specific DNA-binding regulatory proteins (8, 18). The N-terminal region of Cyc8 consists of 10 tandem repeats of a sequence motif termed tetratrico peptide repeat (TPR) (16). TPRs serve as protein-protein interaction domains, and more importantly in the case of Cyc8, TPRs exhibit distinct interaction specificity although they are similar in primary structure (17–20). TPR1, TPR2, and TPR3 contact Tup1, while different combinations of TPR4 to TPR10 mediate recruitment of Cyc8-Tup1 to different promoters (18). Based on these observations it was proposed that the function of Cyc8 is to link Tup1 to distinct, structurally dissimilar, DNA-bound repressor proteins. Consistently with this linker function, derivatives of Cyc8 that contain only the TPR domain are sufficient for repression. On the other hand, the C-terminal domain which comprises more than half of the protein appears to be dispensable (16–18).

Recent genetic data suggested that Cyc8-Tup1 might also play a positive role in transcriptional control. More specifically, activation of the CYC1 gene transcription by the Hap1 transactivator and maximal induction of *SUC2* gene both require functional Cyc8 protein (26, 27). In this report, we present direct evidence that Cyc8-Tup1 indeed plays diverse roles in...
transcriptional regulation. Expression of Cyc8-Tup1 repressible genes is stimulated in response to specific signals, and under these conditions, the fate of the Cyc8-Tup1 co-repressor has been unclear for most of the cases. Our data suggest that this complex can convert to a co-activator of CIT2, a gene encoding a peroxisomal isofrom of citrate synthase that is expressed upon inducing conditions of mitochondrial dysfunction (21). We show that Cyc8-Tup1 is tethered to the CIT2 promoter by interacting with Rtg3, a DNA-binding transactivator of CIT2. Genetic analysis indicates that basal (uninduced) expression of CIT2 is inhibited by Tup1, but its transcriptional activation is mediated by the second component of the complex, the Cyc8 subunit and specifically requires the C-terminal domain of this protein.

The transcriptional activity of Cyc8 was further examined using a synthetic reporter promoter and a LexA-Cyc8 hybrid. Previous studies have shown that LexA-Cyc8 represses transcription by recruiting the Tup1 repressor (18). Here we show that LexA-Cyc8 can activate transcription when Tup1 is absent or when the Tup1 repression is substantially impaired, as it is in a sin4Δ or a rgr1Δ mutant strain. Taken together, these data suggest an inherent potential of the Cyc8-Tup1 co-repressor for transcriptional activation function and establish a dual role (positive and negative) of this complex in transcriptional control.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Growth Conditions—All strains are derivatives of PT5 strain (MATa ura3–52 trp1–63 leu2–3,112 his3 Δ200). The two-hybrid screening was performed in the strain L9FT5, which was constructed by replacing his3 Δ200 with the L9His3 allele. L9His3 promoter contains a single synthetic and perfectly symmetric LexA-binding site in place of the Gen4 UAS (8). cyc9Δ and tup1Δ alleles have been described previously (8). The sin4Δ allele was constructed by inserting HSt1 between the NdeI and Nsil restriction sites. sin4Δ and rgr1Δ strains were generated by one-step gene replacement using linear DNA fragments and were confirmed by Southern analysis.

Standard synthetic media were used; YPD- and YPR-rich media contained 2% glucose or 2% raffinose, respectively. CS minimal media were supplemented with 0.6% casamino acids and glucose or raffinose as the carbon source. Mitochondrial dysfunction was caused by prolonged treatment of cells (~48 h) with 20 μg/ml ethidium bromide in YPD medium. CIT2 induction was monitored in exponential cultures growing in YPR medium. Standard procedure was used for routine yeast transformations, while for high efficiency yeast transformation the TRAPo protocol was followed (22).

Plasmid Constructs—All Cyc8, Tup1, and LexA derivatives expressed from the YCP91 vector and have been described previously (18). Briefly, the centromeric vector YCP91 (TRP1 marked) contains the ADH1 promoter and 5′-untranslated sequence (including the ATG start codon), followed by the SV40 nuclear localization signal and the HA1 epitope from the influenza virus (flue epitope), a polylinker sequence, and the CYC8 termination region (8). The reporter plasmid pACT2 is a URA3 marked multicopy plasmid that expresses the LacZ reporter gene from a minimal promoter consisting of four overlapping LexA-binding sites upstream of the GAL1 TATA box. The Gal4 activation domain-genomic library (used for the two-hybrid screening) was constructed in the pACT2 vector.2 pACT2, a 2μ, LEU2 marked vector contains the ADH1 promoter and transcription start site followed by sequences consisting of nuclear localization signal, the activation domain of Gal4, and polylinker sequence (in which random genomic fragments have been inserted) ending to the termination region of the ADH1 gene.

Two-hybrid Screening for Cyc8-Tup1 Interacting Proteins—LexA-Cyc8, cloned in the YCP91 expression vector, was used to transform L9FT5 along with the LacZ reporter plasmid JK103 (22). L9FT5 yeast transformants appear white on X-Gal indicator plates and are sensitive for growth in low concentrations of 3-amino triazole (0.5–1.0 μM), a competitive inhibitor of His3 enzymatic activity. The pACT2 library was used to transform this strain, and cells were recovered by shaking in selective liquid medium (SC, containing 2% glucose and 2% galactose) for 10 h at 30 °C. Five million independent transformants were scored for growth in minimal media containing 3-amino triazole at a concentration of 20 μM. 200 colonies, scored as positives, were selected (prominent growth in 20 μM 3-amino triazole). Half of them (~96) appeared blue on X-Gal plates. Positive transformants containing pACT2-derived plasmids were rescued in the Escherichia coli KC8 strain (constructed by K. Struhl) based on the ability of the yeast LEU2 gene (pACT2 is a LEU2 marked plasmid) to complement the respective E. coli auxotrophy. 39 of 96 plasmids analyzed reproducibly supported 3-amino triazole resistance and high β-galactosidase activity after recombination into L9FT5 strain. Sequencing analysis revealed seven different ORFs encoding proteins capable of two-hybrid interaction with Cyc8.

GST Interaction Assay—A BamHI-PouII fragment containing Rtg3Δ–68 was cloned in the T7 expression vector pRSETC and was used to direct coupled transcription translation (Promega T7 TNT).3 35S-Labelled Rtg3 protein (10 μl) was hybridized with approximately 2 μg of agarose bead-immobilized GST-Cyc8 protein (18) in a volume of 100 μl containing 20 μM Tris-acetate, pH 7.4, 10% glycerol, 0.2 mM EDTA, 1 mM dithiothreitl, 0.15 M potassium acetate, and 1× complete proteases inhibitors (Boehringer) for 2 h at 4 °C. Following incubation the beads were extensively washed in the above buffer, eluted in SDS-PAGE gel loading buffer, and analyzed by SDS-PAGE.

Results—Whole cell proteins from wild type and cyc8Δ strains were isolated as described previously (24), and protein concentrations were determined by the Bradford assay. A CIT2 promoter region (~530 to +1) was amplified from L9FT5 genomic DNA by polymerase chain reaction, and a BstHIII-AatII fragment of it (106 base pairs) containing the two Rtg1/Rtg3-binding sites, was end-labeled by standard methods using “Klenow” DNA polymerase and purified by Sephadex G-50 chromatography (Amersham Pharmacia Biotech). EMSA reactions were performed with 10 μg of protein extract, 10,000 cpm of CIT2 probe, 4 μg of poly(dI/dC) competitor DNA, 1 mM dithiothreitol, 10 mM Tris-HCl, 100 mM KCl, 2 mM MgCl2, and 5% glycerol in a final volume of 20 μl. They were incubated at 4 °C for 25 min, and samples were separated on a 5% polyacrylamide, 1× TBE gel at 4 °C, 250 V for 2.5 h and visualized by autoradiography.

RNA Analysis—Total cellular RNA was extracted from yeast cells grown in the appropriate medium, using the acid phenol method (25), and was fractionated in 1.4% agarose gels containing 5.5% formalde-hyde. RNA was transferred to nylon membrane and hybridized with 32P-labeled probes generated by nick translation. For CIT2 and TBP probes, polymerase chain reaction fragments containing the entire coding sequence (from ATG to termination codon) of the respective genes were used.

LacZ Assays—β-Galactosidase assays were performed on yeast cultures grown in the appropriate media and harvested during early log phase (Δ abs < 1.0). Cells were washed with 20 mM Tris (pH 7.5), 1.0 mM EDTA in order to disperse the clumpy cyc8 and tup1 cells. LacZ values normalized to Δ abs represent the average of at least three independent transformants, and they are accurate to 20–30%.

RESULTS

Transcriptional Activation by Cyc8—Recent genetic evidence suggested that Cyc8-Tup1 might play a positive role in transcriptional regulation. Mutations in the CYC8 gene adversely affect both Hap1-mediated stimulation of CYC1 and maximal induction of SUC2 transcription (26, 27). Based on these observations, we directly tested whether Cyc8 can stimulate transcription by analyzing the activity of a LexA-Cyc8 hybrid protein on a GAL1-LacZ synthetic reporter that contains a LexA operator upstream of the TATA element. Wild type and isogenic tup1Δ, sin4Δ, and rgr1Δ strains were co-transformed with plasmids carrying genes that express LexA-Cyc8 and the GAL1-LacZ reporter, and transformants were assayed for β-galactosidase activity. As shown in Fig. 1, LexA-Cyc8 represses transcription from the reporter promoter when functional Tup1 is present (wild type strain, line 1). However, in the absence of Tup1 (tup1Δ1), LexA-Cyc8 stimulates transcription by 7-fold (line 2). This result indicates that Tup1 not only actively represses transcription (8) but it also antagonizes the activation potential of Cyc8. This cannot be explained sim-
The Cyc8-Tup1 Protein Complex—If Cyc8-Tup1 acts as a co-activator of complex. formed more efficiently in the context of the Cyc8-Tup1 protein(s). We explored this possibility by seeking such Cyc8-Tup1 interact with promoter-specific DNA-binding activator protein(s). We recovered and sequenced. This selection scheme revealed 39 clones encoding regions from seven different proteins. Noticeably, activation by LexA-Cyc8/Tup1 in these mutant strains is virtually higher (9-fold) than that in the tup1Δ strain. These observations suggest that Cyc8 has the potential to act as a transcriptional co-activator and that this function is performed more efficiently in the context of the Cyc8-Tup1 protein complex.

The Activation Domain of Rtg3 Associates with the Cyc8-Tup1 Protein Complex—If Cyc8-Tup1 acts as a co-activator of certain natural promoters, then it would be expected to directly interact with promoter-specific DNA-binding activator protein(s). We explored this possibility by seeking such Cyc8-Tup1 interacting proteins (activator proteins), using a yeast two-hybrid screen (see “Experimental Procedures”). We generated a strain that expresses HIS3 and LacZ reporter genes under the control of promoters containing a LexA-binding site. This strain was co-transformed with a LexA-Cyc8 expressing plasmid along with a library of random yeast genomic fragments fused to the Gal4 activation domain. Library plasmids supporting high levels of both HIS3 and LacZ reporter genes were recovered and sequenced. This selection scheme revealed 39 positive clones encoding regions from seven different proteins. Consistent with the co-repression function of Cyc8-Tup1, most of these were DNA-binding repressor proteins that inhibit transcription in a Cyc8-Tup1-dependent manner. Interestingly, two independently isolated clones encoded Rtg3, which is the limiting factor for transcription. Both Rtg3–68 (residues 305 to 486) and Rtg3–36 (residues 326 to 486) interacting with a GST-Cyc8 hybrid protein or GST alone immobilized in Sepharose beads. Lane labeled input contains only 20% of the amount of the protein that was incubated with the beads. but not on the column containing GST alone (lane 2), strongly suggesting that Rtg3 directly associates with Cyc8 in the absence of any other yeast protein.

Rtg3 activates the transcription of CIT2, a gene encoding a peroxisomal isofrom of citrate synthase, and probably the transcription of additional genes involved in peroxisome biogenesis (29). Thus, we subsequently explored the function of Cyc8 and Tup1 in the context of the natural CIT2 promoter. Dual Function of Cyc8-Tup1 on CIT2 Transcription—CIT2—Transcription is induced by mitochondrial dysfunction, and this regulatory pathway, through which nuclear gene transcription responds to the functional state of mitochondria, is termed retrograde regulation (24, 30). Both basal expression and retrograde response of CIT2 is mediated by the Rtg3 trans-activator, which is always bound to the CIT2 promoter.

A typical retrograde response of CIT2 gene transcription is shown in Fig. 4A. CIT2 expression is much higher in wild type cells growing under conditions of mitochondrial dysfunction (lane 2), compared with the basal level of expression observed in normally growing cells (lane 1). However, basal expression and retrograde response of CIT2 transcription are dramatically reduced in a strain carrying a chromosomal deletion of CYC8 (lanes 3 and 4), suggesting that CIT2 is positively regulated by Cyc8. On the other hand, basal expression of CIT2 is increased in a tup1Δ strain (lane 5) indicating that CIT2 transcription is yet another target of the Tup1 repression activity. In the tup1Δ strain, retrograde response appears to be comparable, although slightly lower, than in wild type strain (lane 6). Thus, Tup1 inhibits basal expression of CIT2 but it might also be required along with Cyc8 for maximal CIT2 induction. In agreement with this notion, CIT2 expression is fully de-repressed in Tup1 repression defective strains, such as sin4Δ and rgr1Δ (lanes 7 and 8), in which Tup1 is expressed normally. In fact, the expression levels of CIT2 in these mutant strains are comparable with those observed under conditions of mitochondrial dysfunction. Taken together, these results strongly suggest that Cyc8-Tup1 has a dual function on the CIT2 promoter; it inhibits basal transcription, but moreover it acts as a co-activator that mediates retrograde response. It is noteworthy that Rtg3, which is the limiting factor for CIT2 transcription (28), is present at equal levels in wild type, cyc8Δ, and tup1Δ cells growing either at normal or at inducing conditions (28, 29, and data not shown).
Distinct TPR Motifs of Cyc8 Interact with Tup1 and Rtg3—

Two-hybrid assays performed in cyc8Δ and tup1Δ strains indicated that Rtg3 specifically interacts with Cyc8 even in the absence of Tup1, while Tup1 interacts with Rtg3 only in the presence of Cyc8 (data not shown). The TPR domain of Cyc8 mediates protein-protein interactions and was proposed to link specific DNA-binding proteins to Tup1 (18). Thus, we examined whether Rtg3 interacts with specific TPR motifs of Cyc8 by testing various deletion derivatives of Cyc8 for Rtg3 interaction in a two-hybrid assay (Table I and Fig. 5). N175, that contains only three N-terminal TPRs (TPR1 to TPR3), does not activate transcription of the LacZ reporter demonstrating its failure to interact with Rtg3. In contrast, N300 that contains TPR1 to TPR7 strongly interacts with Rtg3 (it activates transcription over 30-fold), indicating that interaction with Rtg3 is mediated by specific TPR motifs, probably TPR4 to TPR7. However, the internally deleted Cyc8 derivative Δ175–281 that lacks TPR4 to TPR7 but maintains TPR8 to TPR10 also interacts with Rtg3 as judged by its activity on the LacZ reporter, which is stimulated over 30-fold. Finally, derivatives such as C560, which comprise the C-terminal domain of Cyc8 but lack TPR sequences, are completely inactive. These data suggest that Rtg3 interacts with at least two independent combinations of TPR motifs, TPR4–TPR7 and TPR8–TPR10. It should be noted that none of these regions overlap with the Tup1 interaction domain, which consists of TPR1 to TPR3 (Ref. 18 and Fig. 5), and it explains how Rtg3 and Tup1 (which do not interact directly) can simultaneously associate with the TPR domain of Cyc8.

To test whether the TPR domain is sufficient to recruit Cyc8-Tup1 to the CIT2 promoter we performed a band shift experiment using whole yeast protein extracts and a probe encompassing two Rtg3/Rtg1-binding sites (Rtg3 binds DNA as a heterodimer with Rtg1, another bHLH/Zip protein, see “Discussion”). In agreement with previous data (29), a stable low mobility complex was detected in the presence of protein extracts derived from a wild type strain (Fig. 6, lane 2). The formation of this complex is dependent on the presence of Cyc8, because extracts from a cyc8Δ strain do not give rise to shifted bands (lane 3). Moreover, ectopic expression of Cyc8 in the cyc8Δ strain restores complex formation (lane 4), and more importantly, the complex is formed even by expressing only the TPR domain of Cyc8 (lane 5). These results strongly suggest that protein-protein interactions mediated by TPR motifs are sufficient to recruit Cyc8-Tup1 to the CIT2 promoter.

The Cyc8 C-terminal Domain Is Essential for Stimulation of CIT2 Transcription—

The TPR domain of Cyc8 provides sufficient Cyc8 function for transcriptional repression by bringing Tup1 to specific DNA-binding proteins, while the C-terminal domain is dispensable. Because we showed that Cyc8-Tup1 activates CIT2, we examined whether recruitment of the complex by the TPR domain is sufficient for positive regulation of CIT2 transcription. For this purpose, derivatives of Cyc8 capable of interacting with both Rtg3 and Tup1, either containing or lacking C-terminal sequences, were expressed in a cyc8Δ strain, and CIT2 mRNA levels were analyzed by RNA blotting. As shown in Fig. 4B and summarized in Fig. 5, Δ175–281, which contains the entire C-terminal domain of the protein, supports wild type levels of CIT2 transcription (lanes 7 and 8). In contrast, N300 and N597 that lack the C-terminal domain are inactive (lanes 3–6), despite their ability to interact with Rtg3 and to complement all previously described cyc8Δ defects (18). Finally, a longer derivative, N816, that contains most of the C-terminal domain is only partially functional (lanes 1 and 2). These results indicate that Rtg3 interaction alone is not sufficient for transcriptional activation of CIT2; normal retrograde response of CIT2 expression requires the C-terminal domain of Cyc8 as well. To our knowledge, retrograde regulation is the only case where a specific function has been assigned to this domain of Cyc8, most likely reflecting the unique regulatory mode of Cyc8-Tup1 action on the CIT2 promoter.

**DISCUSSION**

In this study we provide direct evidence for a dual role of Cyc8-Tup1 in transcriptional control. We found that besides the well established repression activity, which is performed by Tup1, the Cyc8-Tup1 protein complex can also act as a transcriptional co-activator, and this function is predominantly mediated by the Cyc8 protein. When the Tup1 repression activity is impaired, as it is in a sin4 or a rgr1 mutant strain, Cyc8-Tup1 activates an artificial reporter gene, and in response to specific metabolic signals, activates the transcription of the natural CIT2 gene.

Transcription of CIT2 is controlled by Rtg3 and Rtg1, both members of the bHLHZip family of DNA-binding proteins. Recombinant Rtg3 and Rtg1 bind as a heterodimer at two sites within an upstream activation sequence of the CIT2 gene termed UASr (31, 32). Heterologous promoters bearing a UASr, respond to mitochondrial dysfunction in a Rtg1/Rtg3-dependent manner indicating that UASr is sufficient to mediate CIT2 regulation (30). Notably, EMSAs using whole yeast extracts (instead of recombinant Rtg1 and Rtg3 proteins) suggested that additional yeast proteins, probably co-activators or co-repres-
Phenotypes are defined as follows: - , wild type; + , partial function; + + , functionally indistinguishable from cyc8 allele.

Total yeast protein was extracted from strains or from a cyc8 strain expressing either full-length Cyc8 protein or the N300 derivative that contains only TPRs.

The signal(s) that mediate induction of peroxisomal genes as this domain is dispensable for CIT2 function. Under these conditions, CIT2 transcription is derepressed in cells carrying the tup1 mutation. These results suggest that lower CIT2 derepression occurs at even higher levels. This observation further suggests that Cyc8 activation function is better performed in the context of the Cyc8-Tup1 protein complex.

Our data indicate that CIT2 transcription requires the C-terminal domain of Cyc8, and in fact, this is the only case that a function has been assigned to this region. When bound upstream of a test promoter through a heterologous DNA-binding domain this C-terminal region of Cyc8 does not activate transcription (data not shown); therefore it does not function as a typical activation domain, but rather plays a regulatory role. Cyc8 is a phosphoprotein, and specific regions within this C-terminal domain, rich in serine and threonine residues, are potential phosphorylation sites (16). Similarly, Rtg3 contains a serine/threonine-rich region which might also play a regulatory role (28). Thus, it is conceivable that specific modifications of these protein domains, such as phosphorylation or dephosphorylation, may in fact modulate the transcriptional activity of Cyc8-Tup1. Some of these modifications, particularly in the C-terminal domain of Cyc8, are likely to be specific for the retrograde response of CIT2 as this domain is dispensable for the regulation of all other Cyc8-Tup1 repressible genes.

The signal(s) that mediate induction of peroxisomal genes upon mitochondrial dysfunction are presently unknown, and
although several possible models can be envisaged using the available data, the molecular mechanism by which Cyc8-Tup1 is converted from a co-repressor to a co-activator of CIT2 is not yet understood. One model predicts that, upon induction, Tup1 dissociates from the complex thus unmasking Cyc8 activation potential. However, EMSAs performed with protein extracts derived from either normal or mitochondria defective cells detect neither quantitative nor qualitative differences on UASr derived from either normal or mitochondria defective cells dissociates from the complex thus unmasking Cyc8 activation yet understood. One model predicts that, upon induction, Tup1 activate with chromatin (15); thus it is conceivable that Cyc8 might ally interacts with the basic transcription machinery as well as (data not shown). Finally, it is known that Cyc8-Tup1 function- porter under inducing conditions of mitochondrial dysfunction processes.

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