Transactivation by Rtg1p, a Basic Helix-Loop-Helix Protein That Functions in Communication between Mitochondria and the Nucleus in Yeast*

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Rtg1p is a basic helix-loop-helix transcription factor in the yeast Saccharomyces cerevisiae that is required for basal and regulated expression of CIT2, the gene encoding a peroxisomal isoform of citrate synthase. In respiratory incompetent \( \rho^- \) petite cells, CIT2 transcription is elevated as much as 30-fold compared with respiratory competent \( \rho^+ \) cells. Here we provide evidence that Rtg1p interacts directly with a CIT2 upstream activation site (UASr) and that the \( \rho^-/\rho^- \) regulation is not due to a change in the levels of Rtg1p. A fusion protein consisting of the DNA binding domain of Gal4p fused to the NH\(_2\) terminus of the full-length wild-type Rtg1p was able to transactivate an integrated LacZ reporter under control of the Gal4p-responsive GAL1 UAS\(_G\) in a \( \rho^-/\rho^- \)-dependent manner. Other Gal4p fusions to deletions or mutations of Rtg1p indicate that the helix-loop-helix domain is essential for transactivation. Regulated expression of CIT2 also requires the RTG2 gene product. The Gal4-Rtg1p fusion was unable to transactivate the LacZ reporter gene in a strain deleted for RTG2, suggesting that the RTG2 product does not act independently of Rtg1p in the \( \rho^-/\rho^- \) transpositional response.

Recent evidence indicates that the functional state of mitochondria and chloroplasts can influence the expression of nuclear genes (1–4). In Saccharomyces cerevisiae, one such response involves the elevated expression of the nuclear gene CIT2 (encoding a peroxisomal isoform of citrate synthase (5, 6) in cells with dysfunctional mitochondria (7, 8). For example, in respiratory incompetent \( \rho^- \) petites (cells that lack mtDNA), CIT2 mRNA abundance is as much as 30-fold greater than in isochromosomal respiratory competent \( \rho^+ \) cells. This regulation of CIT2 expression appears to be a mechanism for adjusting metabolic interactions between the peroxisomal glyoxylate cycle and the mitochondrial tricarboxylic acid cycle (7, 9).

At least two nuclear genes, RTG1 and RTG2, are required for \( \rho^-/\rho^- \)-responsive transcription of CIT2 (8). Strains with null alleles of either of these genes are unable to use acetate as a sole carbon source, and show growth requirements for glutamate or aspartate, which are phenotypes typical of cells deficient in both the tricarboxylic acid and glyoxylate cycles (10). We have recently found that RTG1 and RTG2 have functions in addition to regulation of CIT2 expression (11); they are together also required for oleic acid-induced expression of genes encoding peroxisomal proteins, as well as for general peroxisome proliferation, which is known to be induced in yeast by oleic acid (12–14). Thus, these genes appear to play a central role in a novel three-way organelle communication between mitochondria, the nucleus and peroxisomes.

RTG1 encodes a 177-amino acid protein (Rtg1p) that is a member of the basic helix-loop-helix (bHLH) family of transcription factors (15, 16). We have found that sequences contained within a 76-bp Mspl-AluI fragment (UASr) in the 5' flanking region of CIT2 are both necessary and sufficient to convey a \( \rho^-/\rho^- \) response to a reporter gene (8). Electrophoretic mobility shift assays (EMSA) using the 76-bp UASr as a probe and extracts from a wild-type strain and a strain deleted for RTG1 reveal an Rtg1p-dependent DNA-protein complex, suggesting that Rtg1p binds to the UASr. However, the UASr, does not contain either an E box (CANNTG) or an N box (CACNAG), which are canonical DNA binding sites recognized by most bHLH proteins (17, 18).

Although RTG2 is required for CIT2 expression, its precise function is unclear. RTG2 encodes a protein containing an HSP70 type of ATP binding domain with similarity to bacterial phosphatases that hydrolyze ppGpp and pppGpp (19). In addition to the absence of any obvious DNA binding motifs in Rtg2p, the EMSA pattern using the CIT2 UASr, as a probe and extracts from cells with the rgt2 null allele is indistinguishable from wild-type extracts, suggesting that the RTG2 product may act indirectly in the regulation of CIT2 expression.

To investigate the role of Rtg1p in transcriptional activation, we have tested the ability of various domains and alleles of RTG1 to activate transcription in \( \rho^- \) and \( \rho^+ \) cells, which have in combination either wild-type or null alleles of the chromosomal RTG1 and RTG2 genes. To assay for transcriptional activation, we have constructed various chimeric protein fusions between the DNA binding domain of the yeast Gal4p transcriptional activator (20, 21) and Rtg1p. This allows the determination of potential transactivation by Rtg1p independent of its intrinsic DNA binding characteristics.

In this paper we show that the full-length Gal4-Rtg1p wild-type fusion protein is able to mediate \( \rho^-/\rho^- \)-responsive transactivation of the reporter gene under UAS\(_G\) control, and that transactivation is dependent on the presence of a wild-type allele of RTG2. We have also used this construct to show that Rtg1p binds directly to the CIT2 UASr. Finally, results are
presenting that Rtg1p interacts, probably via its HLH domain, with another factor, or factors, that are required for the \( \beta^p/\beta^c \) transcripational response.

**MATERIALS AND METHODS**

Yeast Strains and Growth Conditions—The S. cerevisiae strains used in this study are listed in Table I. All strains were derivatives of either COP161 U7 (8) or SFY526 (a gift from Stan Fields and Paul Bertel, SUNY, Stony Brook, NY). All nuclear genotypes exist both as respiratory competent strains containing wild-type mtDNA and respiratory incompetent \( \rho^0 \) derivatives lacking mtDNA. The \( \rho^0 \) derivatives were obtained by several passages through rich dextrose medium containing 20 mg/ml ethidium bromide, then checked for the presence of mtDNA by staining with 4',6-diamidino-2-phenylindole. Cells were grown at 30 °C on YE medium (1% yeast extract, 2% Bacto-peptone) and 3% glycerol (YPG) as a carbon source. Plasmids were selected for by growth on minimal YNB medium (0.67% yeast nitrogen base without amino acids) and 2% dextrose (YNBD) or 2% raffinose (YNBR) supplemented with casamino acids or individual amino acids as required.

Recombinant Plasmids and Site-directed Mutagenesis—Standard molecular biology techniques were used (22). The Escherichia coli strain XL1-Blue (Stratagene, La Jolla, CA) was used to produce recombinant plasmids. The plasmids used in this study are listed in Table I.

The plasmid pRTG1-416 contained a 1.5-kb SphI-PstI genomic fragment containing the entire RTG1 reading frame plus 740 bp of upstream and 300 bp of downstream sequences cloned into pRS416 (CEN, URA3). Site-directed mutagenesis was carried out using the Bio-Rad Mutagen kit in vitro mutagenesis kit.

The pG8T9 plasmid was a gift from Stan Fields and Paul Bertel (Dept. of Microbiology, SUNY, Stony Brook, NY). PCR primers were used to amplify specific fragments of the RTG1 gene for the construction of the 2H series of recombinant pGBT9 plasmids encoding Gal4p DNA binding domain-Rtg1p fusion proteins (Gal4-Rtg1p). All newly constructed plasmids were verified by sequencing using the Sequenase kit (United States Biochemical Corp.).

Production of Anti-sera against Rtg1p—PCR primers were used to construct a pMAL-RTG1 plasmid encoding a full-length Rtg1p-maltose-binding protein fusion. The pMAL-RTG1 plasmid was expressed in E. coli and protein purified following the protocol provided by New England Biolabs for their pMAL-c2 vector system. The purified fusion protein was used to immunize two rabbits following standard procedures (22). Crude serum from these animals was subsequently purified in two steps. First, the serum was presorbed against maltose-binding protein (MBP) coupled to CNBr-Sepharose (Pharmacia Biotech Inc.) to remove MBP-specific antibodies. The serum was then affinity-purified using pMAL-RTG1 fusion protein coupled to CNBr-Sepharose. The Rtg1p-specific antibodies were eluted with 0.5 M glycine HCl, pH 2.3.

Western Blot Analysis—Trichloroacetic acid precipitates of total yeast cell proteins were prepared by pelleting cells from 3 ml of an OD500 = 1 culture grown in YPR following the method of Riezman and Schatz (24). Equal volumes of extract were fractionated on a 12% SDS-PAGE gel, transferred to nitrocellulose and probed with Rtg1p-specific antibodies. The Rtg1p-specific antibodies were eluted with 0.5 M glycine HCl, pH 2.3.

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**RESULTS**

The rtg1-1 Mutant Allele Contains Two Changes Relative to the Wild-type Allele—We previously identified a mutant allele of RTG1 (rtg1-1) that blocked CIT2 expression in both \( \beta^p \) and \( \beta^c \) cells (8). The rtg1-1 allele was cloned by PCR amplification from genomic DNA of the mutant strain. DNA sequence analysis revealed that the mutant allele contained two base pair changes relative to the wild-type allele (Fig. 1A): one was a C → T transition, changing a CCC Pro codon to a TCT Leu codon at amino acid 39 at the end of the first conserved helical domain of the HLH motif; the second was a G → A transition changing a GTG valine codon to an ATG methionine codon at amino acid 112 in the carboxyl-terminal domain of Rtg1p.

To determine whether one or both of these mutations were responsible for the phenotype of the rtg1-1 allele, a complementation test was done in which RTG1, rtg1-1, and the Leu" and Met" mutant alleles were each expressed from a low copy centromeric plasmid. To assay for function of the mutant rtg1 alleles, we used a \( \beta^c \) strain described previously (8) in which a LacZ reporter gene under the control of the CIT2 promoter was integrated into the ura3 gene of the rtg1-1 strain. That strain is defined here as CIT2-LacZ-I (Table I). As shown in Fig. 2, CIT2-LacZ-I cells had a high level of \( \beta^c \)-galactosidase activity...
when transformed with a plasmid containing the wild-type RTG1 gene (pRTG1-416). The single mutation pL112-M constructed restored activity, while the double mutant (prtg1-1), single mutant (pP39-L), and control plasmid (pRS416) all had low activity. These data demonstrate that the mutant phenotype of the original rtg1-1 allele is due largely to the Pro39Leu change at amino acid 39 at the end of helix 1 of Rtg1p.

Rtg1p Levels Are Comparable in r°/rtg1-1, rtg1-1, and Δrtg2 Cells—Since RTG1 is required for basal and retrograde regulation of CIT2 expression, a simple explanation for elevated CIT2 expression in r° cells would be a change in the level of Rtg1p itself. To test this, the level of Rtg1p was monitored in whole cell extracts from r1 and r° derivatives of the wild-type and mutant forms of COP161U7 cells by Western blot analysis. The strains analyzed included the wild-type, rtg1-1 mutant,

| Plasmid               | Based on | Use                        | Comments                        |
|-----------------------|----------|----------------------------|---------------------------------|
| pUCRTG1               | pUC19    | RTG1 disruption             | Deletes aa 1–163 of RTG1\(^a\) |
| pUCRTG2               | pUC19    | RTG2 disruption             | Deletes aa 23–573 of RTG2       |
| pUCrtg1::LEU2         | pUCRTG1  | Construction                | Stratagene                      |
| pRS416                | pRS416   | RTG2 disruption             | 2.4-kb genomic RTG2 fragment    |
| pRTG2–416             | pRTG1–416|                          | Stop codon at aa 273 of RTG2    |
| prtg2::HIS3           | pRTG2–416| RTG2 disruption             | New England Biolabs             |
| pMAL-c2               | pMAL-c2  |                           | RTG1 fused to pMAL              |
| pRTG1–416             | pRTG1–416| Complementation             | rtg1-1 allele                   |
| prtg1–1               | pRTG1–416| Complementation             | Leu\(^{39}\)                    |
| pP39-L                | pRTG1–416| Complementation             | Met\(^{112}\)                   |
| pV112-M               | pRTG1–416| Complementation             |                                 |
| pGBT9                 | pGBT9    | Transactivation             | GAL4-RTG1                       |
| p2H1–177              | pGBT9    | Transactivation             | GAL4-rtg1-1                     |
| p2H26–177             | pGBT9    | Transactivation             | GAL4-RTG1 (aa 26–177)           |
| p2H26–177m            | pGBT9    | Transactivation             | GAL4-rtg1-1 (aa 26–177)         |
| p2H99–177             | pGBT9    | Transactivation             | GAL4-RTG1 (aa 99–177)           |
| p2HP39-L              | pGBT9    | Transactivation             | GAL4-rtg1 (Leu\(^{39}\))        |
| p2HV112-M             | pGBT9    | Transactivation             | GAL4-rtg1 (Met\(^{112}\))       |

\(^a\) aa, amino acid(s).

A

Rtg1p

B

plasmid

pGBT9

p2H1–177

p2H26–177

p2H99–177

p2HP39-L

p2HV112-M

Fig. 1. Panel A, structural domains of Rtg1p including the location of the two point mutations at amino acids 39 (Pro → Leu) and 112 (Val → Met) in the rtg1-1 allele. The basic (b), HLH (H1, L, and H2), and COOH-terminal (C) domains are indicated. The putative protein dimerization HLH motif extends from amino acid 26 to 98. Panel B, coding domains of the GAL4-RTG1 fusion plasmids used in the transactivation assays. PCR was used to insert portions of RTG1 into the EcoRI and BamHI sites of the pGBT9 polylinker fusing Rtg1p to amino acids 1–147 of Gal4p DNA binding domain. The fusions contain an additional 3 amino acids encoded by polylinker sequences.

Fig. 2. A Pro39 → Leu mutation blocks Rtg1p function. CIT2-LacZ\(^r\) cells carrying an integrated LacZ reporter gene under the control of the CIT2 promoter were transformed with a control plasmid (pRS416), or a plasmid carrying the wild-type RTG1 allele (pRTG1–416), or plasmids with the mutant alleles prtg1-1 (prtg1-1), Pro39 → Leu (pP39-L) or Val112 → Met (pV112-M). Cultures were inoculated with a pool of 15–20 transformants and grown to mid-log phase in YNBR supplemented with casamino acids. Extracts were made and specific activity assayed as described under “Materials and Methods.” Extracts were assayed in triplicate for β-galactosidase activity and expressed in the figure as nmol/min/mg of protein.
and two strains described previously (8), harboring a deletion of either RTG1 (Δrtg1) or RTG2 (Δrtg2). Polyclonal Rtg1p-specific antiserum detected a specific band of about 20 kDa, the expected size for the Rtg1p, which was not detected in extracts from the COP1611 Δrtg1 cells (Fig. 3, lanes 1 and 2). Relative to actin, there was no significant difference in the level of the 20-kDa species between ρ⁺ and ρ⁻ wild-type cells (Fig. 3, lanes 3 and 4), suggesting that the large difference in CIT2 expression between these cell types is not due to changes in Rtg1p levels.

We also tested whether Rtg2p could affect CIT2 transcription by modulating the level of Rtg1p. Fig. 3 (lanes 5 and 6) showed that Rtg1p levels were unaffected by the absence of Rtg2p. Finally, the data of Fig. 3 (lanes 7 and 8) showed that in the original rtg1-1 mutant, the nearly complete loss of the specific DNA-protein complex detected in EMSA assays (8), was not the result of any significant instability of the mutant Rtg1p in either ρ⁺ or ρ⁻ cells.

Rtg1p Is Present in a DNA-Protein Complex with the CIT2 UASr—Since the CIT2 UASr does not contain a consensus E box or an N box, it was therefore important to determine whether Rtg1p was actually a part of the DNA-protein complex seen in EMSA of the 76-bp UASr. To do this we took advantage of the construct, p2H1-177, encoding the DNA binding domain of Gal4p fused to full-length Rtg1p (Fig. 1B). This plasmid was constructed for the transactivation studies described in the next section, but proved useful for the analysis of Rtg1p binding to the 76-bp UASr, in EMSA. The Gal4-Rtg1p encoded by p2H1-177 was 327 amino acids long compared to 177 amino acids for endogenous Rtg1p. If the Gal4-Rtg1p was incorporated into the EMSA DNA-protein complex, the complex would run more slowly due to the presence of the larger, recombinant protein. The Gal4-Rtg1 fusion protein was used both to substitute for and to compete with Rtg1p in EMSA.

Whole cell extracts were prepared from SFY526 and SFY526 Δrtg1 strains with and without the p2H1-177 plasmid encoding the full-length wild-type Gal4-Rtg1p fusion. An Rtg1p-dependent band was identified by comparing the 76-bp EMSA from wild-type versus the Δrtg1 strain (Fig. 4, lanes 1 and 3). When extracts from cells transformed with p2H1-177 encoding the full-length fusion protein were used, a larger, slower migrating complex was formed indicating the binding of the larger Gal4-Rtg1p. In the Δrtg1 background only the slower migrating complex containing the fusion protein was present (Fig. 4, lane 4). In the wild-type background, however, both the endogenous Rtg1p and larger Gal4-Rtg1p-dependent complexes were present (Fig. 4, lane 2) indicating that the Gal4-Rtg1p was able to compete with the endogenous Rtg1p for the DNA template.

Rtg1p Can Mediate ρ⁺/ρ⁻-Dependent Transactivation of a Reporter Gene when Fused to the Gal4p DNA Binding Domain—To assay the ability of the Rtg1p to activate transcription, various chimeric plasmids were constructed encoding fusion proteins containing the DNA binding domain of Gal4p fused to the NH₂ terminus of the full-length and truncated versions of Rtg1p (Fig. 1B). The base plasmid was pGBT9 (GenBank™ accession number U07646), a multi-copy 2 μm plasmid carrying TRP1 as a selectable marker and sequences encoding amino acids 1–147 of Gal4p under a constitutive promoter (27, 28). These constructs were transformed into ρ⁺ and ρ⁻ SFY526 cells. Carried an integrated LacZ reporter under the control of a GAL1-responsive GAL1 UASr promoter (29). The Gal4p DNA binding domain would direct binding of the fusion protein to the UASr, so that individual domains of Rtg1p could be tested for their ability to transactivate the LacZ reporter independent of their ability to bind to the CIT2 UASr. In addition, any accessory proteins required for gene activation would be available to interact with or modify Gal4-Rtg1p.

To ensure that the various chimeric plasmids produced stable fusion proteins, whole cell extracts from transformed SFY526 cultures carrying each of the plasmids were fractionated on SDS-PAGE, transferred to nitrocellulose, and probed with polyclonal antiserum raised against Rtg1p. All of the Gal4-Rtg1p fusions described here accumulated to comparable levels regardless of nuclear or mitochondrial background, and were present at higher levels relative to endogenous Rtg1p (not shown).

SFY526 cultures were grown in selective raffinose medium to maintain the GAL4-RTG1 plasmids. Extracts were prepared from log phase cultures and assayed for β-galactosidase activity. Fig. 5A presents a summary of these data. The full-length fusion construct (p2H1-177) not only activated expression of the LacZ reporter but did so in a ρ⁺/ρ⁻-dependent fashion; i.e. β-galactosidase activity was about 2-fold higher in ρ⁺ than in ρ⁻ cells. This difference, however, was much less than the ρ⁺/ρ⁻ transcriptional response of the endogenous CIT2 gene (7, 8). The lower magnitude of the retrograde response using the Gal4-Rtg1p transactivators may be inherent in the heterologous system or, as suggested by the data below, may reflect competition for endogenous components important in the retrograde signaling pathway.

The p2H26-177 construct deleted for the putative basic DNA binding domain of Rtg1p (amino acids 1–25) was considerably more effective in transactivation of the LacZ reporter gene and showed a somewhat greater ρ⁺/ρ⁻ response (3-fold) than the full-length, wild-type construct. A likely explanation for the higher overall activity of p2H26-177 is that in the absence of
Leu39 mutation as the one accounting largely for the mutant phenotype of the original RTG1 disruption strain. As shown in Fig. 5, the putative Rtg1p DNA binding domain, there was less competition with the UASr for the Gal4-Rtg1 fusion protein by endogenous Rtg1p DNA targets.

Cells transformed with the p2H99-177 plasmid encoding just the carboxyl-terminal domain of Rtg1p fused to the Gal4p DNA binding domain had no activity above background (Fig. 5A). Western blot analysis indicated that the fusion protein encoded by this construct was, nevertheless, abundant (data not shown). Thus, the COOH-terminal portion of Rtg1p is not likely to be a transcriptional activator region of Rtg1p that acts independently of the HLH domain.

The full-length mutant form of Rtg1p fused to Gal4p (p2H1-177m) did not transactivate the LacZ reporter in a wild-type SFY526 background (Fig. 5A). The construct carrying the Leu189 mutation (p2HP39-L) was also unable to activate transcription of the LacZ reporter. However, the fusion construct carrying the Met142 mutation (p2HV122-M) transactivated in a p10/p12-dependent fashion at a level similar to the full-length wild-type construct. These results support the in vivo complementation experiments shown in Fig. 2 that identified the Leu189 mutation as the one accounting largely for the mutant phenotype of the original rtg1-1 allele.

Gal4-Rtg1p Is a Potent Transactivator in a Strain Deleted for Endogenous RTG1—To eliminate possible competition for factors required for transcriptional activation that might interact with the endogenous Rtg1p and thus skew the results with the GAL4-RTG1 fusion constructs, a null allele of RTG1 (Δrtg1) was constructed in strain SFY526. Activation of the GAL1-LacZ reporter gene by the various GAL4-RTG1 constructs was then tested in p10 and p12 derivatives of the Δrtg1 strain. As shown in Fig. 5B, β-galactosidase activity in both p10 and p12 cells transformed p2H1-177 and p2H26-177 was much higher than in the wild-type SFY526 background. (Note the change in scale of the β-galactosidase activity in Fig. 5, panel A versus B). The COOH-terminal domain of construct p2H99-177, however, was still unable to activate expression of the LacZ reporter gene in either p10 or p12Δrtg1 cells.

Surprisingly, the proteins encoded by the three mutant constructs carrying the Leu189 mutation (p2H1-177m, p2HP39-L, and p2H26-177m), which were inactive in wild-type SFY526 cells, were potent transactivators in the SFY526 Δrtg1 strain and showed a p10/p12 retrograde response even greater than the wild-type construct, p2H1-177. Among bHLH proteins, the Pro89 at the end of helix 1 is a highly conserved residue (8). A change to Leu could affect the conformation of the HLH motif and alter subsequent protein-protein interactions. However, in some cases leucine is permitted at this location, as in myogenin (30). This may explain why the mutant GAL4-RTG1 fusions (p2H1-177m, p2H26-177m, and p2HP39-L) expressed in the Δrtg1 background were effective transactivators. The mutant proteins may be able to interact with other factors required for transactivation but could not effectively compete with wild-type Rtg1p for them or for the endogenous UASr sites.

The Gal4-Rtg1p Fusion Protein Is Not a Strong Transactivator when Expressed in Cells Deleted for RTG2—Δrtg2 is required for efficient transactivation by Gal4-Rtg1p (8). However, the pattern and intensity of the EMSA using the 76-bp UASr was not as that Rtg2p influences transcription independent of Rtg1p, or that Rtg2p affects a putative Rtg1p-dependent DNA-protein complex in a manner that does not alter the binding of factors to the UASr. We have examined this point using the Gal4-Rtg1p transactivators in a Δrtg2 and a Δrtg1 Δrtg2 strain, whereby transactivation by the Gal4-Rtg1p does not require binding to the UASr. Fig. 6 compares the transactivation activity of p2H1-177, p2H1-177m, and p2H26-177 constructs in wild-type, Δrtg1, Δrtg2, and Δrtg1 Δrtg2 strains. The data show that β-galactosidase activity in both the p10 and p12 strains is very low in the Δrtg2 and Δrtg1 Δrtg2 backgrounds when compared with the wild-type and Δrtg1 strains, respectively. These findings suggest that Rtg2p regulates CIT2 transcription by affecting Rtg1p, or associated proteins, rather than via an independent interaction with the UASr. Both p10 and p12 activities in the Δrtg1 strain were in the range of the control Gal4 DNA binding domain plasmid, pGB79, making it difficult to assess a p10/p12 response with any certainty. However, the higher activity in the Δrtg1 Δrtg2 strain clearly showed a p10/p12 response for all three constructs, although the -galactosidase activity in panels A–D.

**DISCUSSION**

Rtg1p Is Not Limiting in the p10/p12-responsive Regulation of CIT2 Transcription—The bHLH transcription factor encoded by the RTG1 gene is required for both basal expression of the CIT2 gene as well as for its elevated expression in cells with dysfunctional mitochondria, such as in p12 petites (8). One possible explanation for the dramatic difference in CIT2 expression between p10 and p12 cells is a change in the level of Rtg1p...
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The analysis of point and deletion mutants of Rtg1p is also essential. In the absence of the endogenous Rtg1p, competition for X in reaction 1 is reduced or eliminated accounting for the marked increase in LacZ expression (reaction 5). Removal of the HLH domain in p2H9B–177 prevents any interaction with X (reaction 2 or 5). The Leu39 mutant fusion construct would be unable to compete with endogenous Rtg1p for X (reactions 2 and 5 versus reaction 1). However, in the absence of endogenous Rtg1p, some complex formation would occur to allow LacZ expression. Recently, we have identified a third gene, RTG3, that is required for ρ+/ρ0 CIT2 regulation, and experiments are in progress to determine whether it encodes "X" or some other factor.

RTG2 Potentiates Transactivation by Gal4-Rtg1p—The levels of CIT2 mRNA in Δrtg1 and Δrtg2 strains are at the threshold of detection in both ρ− and ρ+ cultures (8). It was therefore impossible to determine epistasis between RTG1 and RTG2 by analysis of CIT2 expression. The dramatic decrease in transactivation by the Gal4-Rtg1 fusion construct in the Δrtg2 backgrounds (Fig. 6) clearly shows that the Rtg1p requires the RTG2 product in either ρ− or ρ+ cells for full activity. It also suggests that RTG2 influences CIT2 transcription through Rtg1p rather than via an independent pathway. Extracts from cells deleted for RTG2 are still able to form the RTG1-dependent EMSA complex with the 76-bp UASr, (8). Therefore, RTG2 appears not to influence complex formation but may act to modify one or more of the components associated with the CIT2 UAS, transcriptional complex, perhaps in response to signals from mitochondria.

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