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RTG-dependent Mitochondria-to-Nucleus Signaling Is Regulated by *MKS1* and Is Linked to Formation of Yeast Prion [URE3]

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An important function of the *RTG* signaling pathway is maintenance of intracellular glutamate supplies in yeast cells with dysfunctional mitochondria. Herein, we report that *MKS1* is a negative regulator of the *RTG* pathway, acting between Rtg2p, a proximal sensor of mitochondrial function, and the bHLH transcription factors Rtg1p and Rtg3p. In *mks1*Δ cells, *RTG* target gene expression is constitutive, bypassing the requirement for Rtg2p, and is no longer repressible by glutamate. We show further that Mks1p is a phosphoprotein whose phosphorylation pattern parallels that of Rtg3p in response to activation of the *RTG* pathway, and that Mks1p is in a complex with Rtg2p. *MKS1* was previously implicated in the formation of [URE3], an inactive prion form of a negative regulator of the nitrogen catabolite repression pathway, Ure2p. *rtg*Δ mutations induce [URE3] and can do so independently of *MKS1*. We find that glutamate suppresses [URE3] formation, suggesting that the Mks1p effect on the formation of [URE3] can occur indirectly via regulation of the *RTG* pathway.

INTRODUCTION

Cells are able to monitor and respond to the functional state of their organelles. In animal cells, for example, compromises in mitochondrial function or certain external cues can lead to increased expression of genes encoding components of the mitochondrial oxidative phosphorylation apparatus and, in some instances, lead to a general increase in the biogenesis of mitochondria (Lunardi and Attardi, 1991; Scarpulla, 1997; Biswas *et al.*, 1999; Heddi *et al.*, 1999; Murdock *et al.*, 1999; Wu *et al.*, 1999; Amuthan *et al.*, 2001). These events are controlled, in part, by transcriptional activators and coactivators whose targets include nuclear genes encoding mitochondrial proteins.

Yeast cells also respond to mitochondrial dysfunction by altering the expression of a subset of nuclear genes (Parikh *et al.*, 1987, 1989). This response, called retrograde regulation, functions to better adapt cells to the mitochondrial defects. In derepressed, respiratory-deficient cells, such as those that have lost their mitochondrial DNA (ρ^o petites), the expression of genes involved in anaplerotic pathways, small molecule transport, peroxisomal activities, and stress responses is up-regulated (Liao *et al.*, 1991; Liu and Butow, 1999; Hallstrom and Moye-Rowley, 2000; Traven *et al.*, 2000; Epstein *et al.*, 2001). In many cases, these changes in gene expression reflect activities that would compensate for the block in the tricarboxylic acid (TCA) cycle caused by the respiratory defect. Expression of a number of these retrograde responsive genes, such as *CIT2*, *DLD3*, and *PDH1* encoding, respectively, a glyoxylate cycle isoform of citrate synthase, a cytosolic D-lactate dehydrogenase, and a protein involved in propionate metabolism, is controlled by *RTG1*, *RTG2*, and *RTG3.* Rtg1p and Rtg3p are basic helix-loop-helix transcription factors (Jia *et al.*, 1997), and Rtg2p is a cytoplasmic protein with an N-terminal ATP-binding domain similar to that of the actin/sugar kinase/hsp70 superfamily (Bork *et al.*, 1992). Rtg2p plays a pivotal role in the retrograde pathway, because it is both a sensor of the functional state of mitochondria and is required for the activation of *RTG*-dependent gene expression by promoting the cytoplasmic-to-nuclear translocation of Rtg1p and Rtg3p (Sekito *et al.*, 2000). In addition, Rtg2p is required for the partial dephosphorylation of Rtg3p associated with its nuclear accumulation.

The retrograde pathway senses the functional state of mitochondria via the level of glutamate (Liu and Butow, 1999; Sekito *et al.*, 2000; Epstein *et al.*, 2001). Because glutamate is a potent repressor of *RTG*-dependent gene expres-

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sion, the retrograde pathway is likely to be important for glutamate homeostasis. In cells with compromised or dysfunctional mitochondria, the expression of *CIT1*, *ACO1*, *IDH1*, and *IDH2* (genes encoding the enzymes catalyzing the first three steps in the TCA cycle that lead to the production of α-ketoglutarate, the direct precursor of glutamate) is controlled by the *RTG* genes (Liu and Butow, 1999); in cells with robust mitochondrial function, expression of these genes is under control of the HAP transcription complex (Forsburg and Guarente, 1989; Rosenkrantz *et al.*, 1994). A connection between the retrograde pathway and nitrogen metabolism has recently been uncovered by the finding that the target of rapamycin (TOR) kinase pathway in yeast also involves the *RTG* genes (Komeili *et al.*, 2000; Shamji *et al.*, 2000). The TOR kinase pathway promotes the formation of a complex between Gln3p, a transcription factor that controls the expression of genes required for the utilization of poor nitrogen sources (Mitchell and Magasanik, 1984; Courchesne and Magasanik, 1988; Coschigano and Magasanik, 1991; Blinder *et al.*, 1996), and Ure2p, a negative regulator of Gln3p (Beck and Hall, 1999). When the TOR kinase pathway is inhibited by rapamycin, or when cells are grown on a poor nitrogen source such as urea, Rtg1p and Rtg3p translocate from the cytoplasm to the nucleus in an Rtg2p-dependent manner to activate target gene expression (Komeili *et al.*, 2000).

Herein, we report the identification of a new regulatory gene in the *RTG* pathway, *MKS1.* Our data show that *MKS1*, originally described as a regulator of the Ras-cAMP pathway (Matsuura and Anraku, 1993), is a negative regulator of *RTG*-dependent gene expression. *MKS1* was also known as *LYS80*, a negative regulator of lysine biosynthesis (Feller *et al.*, 1997) and, more recently, proposed to regulate the nitrogen catabolite repression (NCR) pathway via its effects on the negative regulator of that pathway, Ure2p (Edskes *et al.*, 1999). Mks1p was reported to be required for the generation of [URE3], the inactive prion form of Ure2p (Edskes and Wickner, 2000). We find that glutamate is a potent repressor of [URE3] formation, and that inactivation of the *RTG* pathway, which causes a decrease in the intracellular glutamate supply, induces [URE3].

MATERIALS AND METHODS

Media, Growth Conditions, Strains, and Constructs

Cells were grown at 30°C in YPR medium (YP + 2% raffinose), YNBR + cas medium (0.67% yeast nitrogen base containing 1% casamino acids and 2% raffinose), or YNBD (0.67% yeast nitrogen base and 2% dextrose). Glutamate was added at concentrations indicated in the text and figures. Where required, media were supplemented with 30 mg/l leucine, 30 mg/l lysine, 20 mg/l uracil as described (Rose *et al.*, 1990). Ureidosuccinic acid was added at a final centration of 200 μg/ml. Rapamycin was dissolved in 10% Tween 20/90% ethanol and added at a final concentration of 0.2 μg/ml.

The ρ^+ and ρ^o derivative of strain PSY142 (*MATα leu2 lys2 ura3*) and their *rtg2*Δ and *rtg3*Δ derivatives have been previously described, as well as the derivatives of these strains containing a single chromosomal copy of a *CIT2-lacZ* reporter gene (Liu and Butow, 1999; Sekito *et al.*, 2000). Similar derivatives of strain MLY42 (*MATα ura3 leu2*), a Σ1278b derivative, and CC34 (*MATα trp1 ade2 leu2 his3 ura2::HIS3*) were constructed as done for strain PSY142. The $LEU2^+$ derivatives of MLY42 were obtained by transforming cells with YDp-LEU2 digested with *Bam*HI. mks1Δ derivatives of these strains were constructed by replacing the *MKS1* locus (from −66 to +1595) with *URA3* or *LEU2*. The various *ura2*Δ derivatives of these strains used to detect ureidosuccinic acid $(USA)^+$ colonies (see below) were obtained by replacing the *URA2* locus (from +45 to +6594) with the kanMX4 cassette (Wach *et al.*, 1994). Strain RBY453 (*MAT**a** ura2 met*) was used as a tester in crosses for the genetic properties of USA^+ cells. The original *mks1* mutants were obtained by screening ethyl methanesulfonate-mutagenized PSY142 *rtg2*Δ ρ^+ cells containing an integrated *CIT2-lacZ* reporter gene for the appearance of dark blue colonies on solid YNBR + cas medium containing X-Gal.

pRS416Mks1-GFP was constructed by polymerase chain reaction (PCR) amplification of the *MKS1* coding and 5′-flanking regions from −1007 to +1752 by using the oligonucleotides 5′-TAGGAGCTCGGGGATGCCCAAGTTTAT-3′ and 5′-TTACTCGAGCTATTCGCCCTCCATTACTC-3′ (restriction sites used for cloning are underlined). The oligonucleotides 5′-CGAGGTACCTTACTAAGTTAAATAAATCAGATA-3′ and 5′-TTACTCGAGCTATTCGCCCTCCATTACTC-3′ were used to PCR amplify 550 base pairs of the 3′-untranslated region of *MKS1*. The PCR products were cleaved with the appropriate restriction enzymes, and a 727-base pair *Xho*I-*Kpn*I fragment containing the coding region of a bright green version of green fluorescent protein (GFP) was cloned into the *Xba*I-*Bam*HI site of the yeast centromere plasmid pRS416. Transplacement of Mks1-GFP into the genomic *MKS1* locus was conducted as described by Sekito *et al.* (2000). Transplacement of Rtg3-GFP into the genomic *RTG3* locus was described previously (Sekito *et al.*, 2000).

Assays

β-Galactosidase assays were carried out as described (Liu and Butow, 1999). Assays were conducted in triplicate and independent experiments were carried out two or three times. Trichloroacetic acid precipitation of total yeast cell proteins, treatment with calf intestinal phosphatase, SDS-PAGE, and immunodetection of proteins by Western blotting were carried out as described (Sekito *et al.*, 2000). Rtg3p was detected with anti-Rtg3p antiserum (Sekito *et al.*, 2000). Mks1p was detected as a C-terminal, triple hemagglutinin (HA)-tagged derivative, $Mks1p(HA)_3$, in cells from a construct cloned into the plasmid pRS416 by using a monoclonal anti-HA antibody (12CA5). Dephosphorylation of whole-cell extracts was performed by incubation of neutralized TCA precipitates at 30°C for 30 min, with 5 U of calf intestinal phosphatase or 80 U of λPPase. For Northern blot analysis, cells were grown to OD 0.8 in YNBR + cas medium. RNA extraction and Northern blot analysis were performed as described (Liu and Butow, 1999). A 3.3-kb *MKS1* DNA probe was labeled using a Random primed DNA labeling kit (Roche Applied Science, Indianapolis, IN). The Rtg1p, Mks1p, and Rtg3p-GFP fusion proteins were expressed from constructs transplaced into the respective *RTG1* and *RTG3* chromosomal loci and their visualization in cells by immunofluorescence microscopy was carried out as described (Sekito *et al.*, 2000). To assay for the USA^+ phenotype, the various strains noted in the text containing a *ura2*Δ mutation were individually cultured to saturation in YPD (1% yeast extract, 2% bacto peptone, and 2% dextrose). Between 10^6 and 10^7 cells were plated on YNBD medium supplemented with appropriate amino acids, and 200 μg/ml USA. The number of USA^+ colonies per plate was determined after 5 d of incubation at 30°C. Genetic analyses of the USA^+ phenotype were carried out by standard procedures. Guanidine curing of USA^+ cells was carried out by plating cells from USA^+ colonies on YPD plates containing 5 mM guanidine HCl. After 3 d growth at 30°C, the cells were replated on YPD-5 mM guanidine HCl plates, grown again for 3 d, and then scored for USA^+ phenotype as described above.

Coimmunoprecipitation Experiments

Preparations of cell extract for coimmunoprecipitation were done as previously described (Sekito *et al.*, 2000) except that solution A containing phosphatase inhibitors (0.1 mM Na_3VO_4, 50 mM NaF,

and 5 mM sodium pyrophosphate). Five hundred-microliter cell extracts (300 ng/μl protein) were incubated at 4°C for 2 h with monoclonal anti-myc antibody (9E10), after which 100 μl of a 50% slurry of protein G-Sepharose (Roche Applied Science) was added for each reaction then further incubated at 4°C for 2 h. The immune complexes bound to the Sepharose beads were released by boiling in SDS-PAGE sample buffer after washing five times with solution A. The released immune complexes were analyzed by Western blotting with a monoclonal anti-myc antibody or monoclonal anti-HA antibody. A construct encoding Mks1p(myc)$_3$ was transplaced into the chromosomal *MKS1* locus. Rtg2p(HA)$_3$ and Rtg3p(HA)$_3$ were expressed in cells from constructs cloned into the plasmid pRS416. A similar protocol was used in the reciprocal coimmunoprecipitation experiments with Mks1(HA)$_3$ and myc-tagged Rtg2p and Rtg3p derivatives.

RESULTS

MKS1 Mutations Bypass the Requirement for Rtg2p in Rtg1p-Rtg3p Target Gene Expression

To identify additional regulatory genes in the retrograde pathway, we performed a suppressor screen for mutations that would allow expression of an integrated *CIT2-lacZ* reporter gene in *rtg2*Δ cells. This screen yielded 14 recessive, single-gene mutations that fell into two complementation groups. Subsequent screening of the mutants transformed with a wild-type yeast centromeric-based plasmid library revealed that the high level of *CIT2-lacZ* expression in the mutagenized *rtg2*Δ cells of one of the complementation groups could be restored to a low level of expression by *MKS1*. Details of the other complementation group will be reported elsewhere.

Sequencing of two of the *mks1* mutant alleles showed that each contained a chain termination codon (our unpublished data). To verify the mutant phenotype and to characterize more generally the effects of *MKS1* on *RTG*-dependent gene expression, we examined the effects of an *mks1*Δ mutation on the expression of an integrated *CIT2-lacZ* reporter gene in wild-type and *rtg2*Δ or *rtg3*Δ derivatives of ρ^+ and ρ^o cells of strain PSY142 (Figure 1). Typical of the retrograde response, reporter gene expression was greater in respiratory-deficient ρ^o cells, and expression in both ρ^+ and ρ^o cells was completely dependent on *RTG2* and *RTG3*. However, the block in reporter gene expression in *rtg2*Δ cells was not only suppressed by the *mks1*Δ mutation but also in both the ρ^+ and ρ^o rtg2Δ mks1Δ double mutants, expression levels were significantly greater than observed in otherwise wild-type ρ^o cells. In contrast, the *mks1*Δ mutation was unable to suppress the block in reporter gene expression in *rtg3*Δ cells, suggesting that suppression of the *rtg2*Δ mutation by *mks1*Δ does not occur by activation of some alternative pathway. Finally, the *mks1*Δ mutation alone resulted in high levels of *CIT2-lacZ* expression, exceeding even that observed in ρ^o cells.

MKS1 Negatively Regulates RTG-dependent Gene Expression Upstream of Rtg1p-Rtg3p

To investigate the cellular and molecular events associated with *MKS1* regulation of the *RTG* pathway, we first examined the intracellular localization of functional derivatives of Rtg3p and Rtg1p, each tagged at their C terminus with GFP and transplaced into the respective *RTG3* and *RTG1* chromosomal loci. In agreement with our previous findings (Sekito *et al.*, 2000), these transcription factors were predominantly cytoplasmic in wild-type ρ^+ cells in which *CIT2* expression is low (Figure 2A). In *mks1*Δ and *mks1*Δ *rtg2*Δ double mutant ρ^+ cells, however, they were predominantly nuclear, consistent with the high level of *CIT2-lacZ* expression in those mutants. Nuclear localization of Rtg3p was confirmed by colocalization of nuclear DNA stained with 4′,6′-diamino-2-phenylindole (our unpublished data).

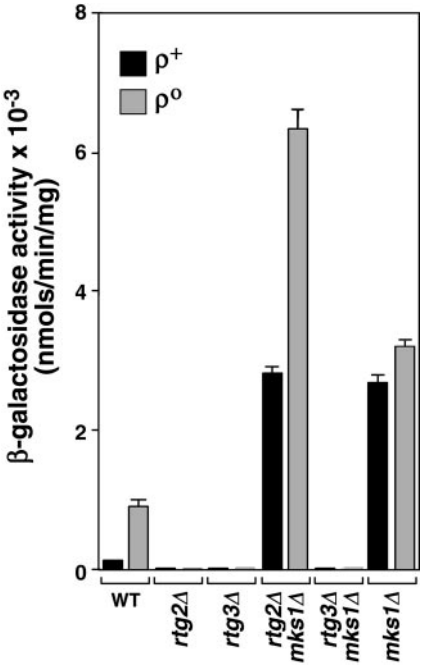

Figure 1. Inactivation of *MKS1* reverses the block in *CIT2* expression in *rtg2*Δ cells. β-Galactosidase activities were determined in triplicate in whole-cell extracts of the indicated strains grown to midlog phase in YNBR + cas medium. Each strain contained a *CIT2-lacZ* reporter gene integrated at the *CIT2* locus.

Previous work established that in wild-type ρ^+ cells, the cytoplasmic form of Rtg3p is phosphorylated at multiple sites (Sekito *et al.*, 2000), and that activation of *RTG*-dependent gene expression in respiratory-deficient ρ^o cells leads to the partial dephosphosphorylation of Rtg3p accompanying its nuclear accumulation (together with nuclear accumulation of Rtg1p). Furthermore, in *rtg2*Δ mutant cells in which *CIT2* expression is undetectable, not only is the nuclear accumulation of Rtg3p and Rtg1p blocked but also Rtg3p becomes hyperphosphorylated relative to its phosphorylation state in wild-type ρ^+ cells. We therefore determined whether the inactivation of *MKS1* in ρ^+ wild-type and *rtg2*Δ cells also affected the phosphorylation state of Rtg3p. Using polyclonal antiserum raised against recombinant Rtg3p to detect Rtg3p by Western blotting, we found that in wild-type and *rtg2*Δ cells, introduction of the *mks1*Δ mutation resulted in an extensive dephosphorylation of Rtg3p (Figure 2B).

RTG-dependent gene expression is also regulated by glutamate via a negative feedback loop, whereby low levels of glutamate activate the *RTG* pathway, and high levels of

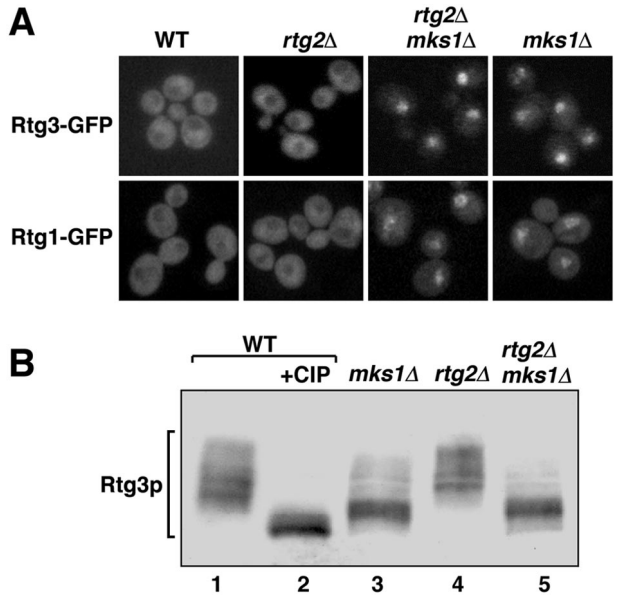

Figure 2. Effects of inactivation of *MKS1* in wild-type and *rtg2*Δ cells on Rtg1p and Rtg3p. (A) Subcellular localization of C-terminal GFP derivatives of Rtg1p and Rtg3p in ρ^+ wild-type (WT) and mutant derivatives of strain PSY142. Constructs encoding the GFP derivatives were transplaced into the respective *RTG1* and *RTG3* loci. (B) Rtg3p is dephosphorylated in *mks1*Δ cells grown in YNBD + cas medium. Rtg3p was detected by Western blotting with a polyclonal anti-Rtg3p antibody raised against recombinant Rtg3p (Sekito *et al.*, 2000). Lane 2 shows that the mobility of Rtg3p collapses to a faster migrating species when an extract of wild-type cells was pretreated with 5 U of calf intestinal phosphatase (CIP).

glutamate inhibit it (Liu and Butow, 1999; Sekito *et al.*, 2000). This regulation reflects one of the primary functions of the *RTG* pathway: to ensure that adequate supplies of glutamate are available for biosynthetic reactions. When the *RTG* pathway is debilitated, for instance, in *rtg2*Δ or *rtg3*Δ strains with compromised or dysfunctional mitochondria, these strains are glutamate auxotrophs (Liao and Butow, 1993; Jia *et al.*, 1997; Liu and Butow, 1999). We therefore wished to determine whether Mks1p also functions in glutamate regulation of the *RTG* pathway.

We first tested the effect of the *mks1*Δ mutation on the glutamate auxotrophy of *rtg2*Δ and *rtg3*Δ cells. As shown in Figure 3A, the inability of *rtg2*Δ cells to grow in medium lacking glutamate was reversed by introduction of the *mks1*Δ mutation; *rtg3*Δ cells, however, remained glutamate auxotrophs when *MKS1* was inactivated. These observations are consistent with the restoration of *CIT2* expression by the *mks1*Δ mutation in *rtg2*Δ, but not in *rtg3*Δ cells. In the converse experiment (Figure 3B), glutamate repression of *CIT2-lacZ* reporter gene expression in ρ^+ cells grown in minimal dextrose medium was not only largely attenuated in *mks1*Δ and *mks1*Δ *rtg2*Δ cells but also the *mks1*Δ mutation by itself resulted in an even greater level of reporter gene expression than was observed in wild-type cells grown in medium lacking glutamate. Accompanying the strong glutamate repression of *CIT2-lacZ* expression was an increased phosphorylation of Rtg3p, an effect that was also suppressed by the *mks1*Δ mutation alone or in the presence of an *rtg2*Δ mutation (Figure 3C). Collectively, these data strongly support the conclusion that *MKS1* is a negative regulator of the *RTG* pathway and suggest that Mks1p acts downstream of Rtg2p, but upstream of Rtg1p-Rtg3p.

Mks1p Is a Phosphoprotein

To investigate further the relation between *MKS1* and *RTG*-dependent gene expression, we constructed a chimeric gene encoding a triple HA_3 tag fused to the C terminus of Mks1p. This HA-tagged version of Mks1p [Mks1p$(HA)_3$], which can complement the *mks1*Δ mutation, was used to follow the fate of Mks1p in a variety of cell types and growth conditions. Immunoblot analysis of Mks1p$(HA)_3$ in extracts of ρ^+ cells grown in medium containing 0.2% glutamate showed multiple electrophoretic forms that collapsed to a single, faster migrating species upon pretreatment of the extract with λ phosphatase, indicating that Mks1p is a phosphoprotein (Figure 4A). A comparison of ρ^+ versus ρ^o cells grown in rich medium shows that Mks1p$(HA)_3$ is more phosphorylated in ρ^+ than in ρ^o cells (Figure 4B, lanes 1 and 2). Similarly, the addition of 0.2% glutamate to the growth medium resulted in an increase in the phosphorylation of Mks1p$(HA)_3$ (compare Figure 4B, lanes 3 and 4). In *rtg2*Δ cells, Mks1p$(HA)_3$ was much less abundant, but that which could be detected was hyperphosphorylated (Figure 4B, lane 5). In contrast, Mks1p$(HA)_3$ was abundant and dephosphorylated in *rtg3*Δ cells (Figure 4B, lane 6). These results show that Mks1p is a phosphoprotein whose phosphorylation pattern changes in response to signals that affect *RTG*-dependent gene expression.

Rapamycin Treatment Results in Dephosphorylation of Mks1p and Rtg3p

In addition to changes in the functional state of mitochondria, the intracellular localization of Rtg1p and Rtg3p has been shown to be affected by the quality of the nitrogen source in the medium (Komeili *et al.*, 2000). Thus, in cells grown in medium containing poor nitrogen sources, *CIT2* expression is activated and Rtg1p and Rtg3p accumulate in the nucleus. Activation of *CIT2* expression and nuclear accumulation of these transcription factors was also shown to occur when cells were treated with rapamycin, an inhibitor of the TOR kinase pathway. We first confirmed that rapamycin addition to PSY142 cells grown in medium containing glutamate resulted in the nuclear localization of an Rtg3p-GFP derivative (Figure 5A). Under these conditions of treatment of cells with rapamycin there was a clear, partial dephosphorylation of Rtg3p (Figure 5B), consistent with our previous observations (Sekito *et al.*, 2000) that activation of *CIT2* expression and nuclear localization of Rtg3p (and Rtg1p) is associated with a partial dephosphorylation of Rtg3p. Mks1p also was partially dephosphorylated when cells were treated with rapamycin, but in contrast to Rtg3p, an Mks1-GFP derivative remained cytoplasmic in the presence of rapamycin or by the exclusion of glutamate from the growth medium. The similarity in the phosphorylation behavior of Mks1p and Rtg3p (Sekito *et al.*, 2000) to conditions that affect *RTG*-dependent gene expression suggests that these proteins may be modified by the same kinase/phosphatase pathway.

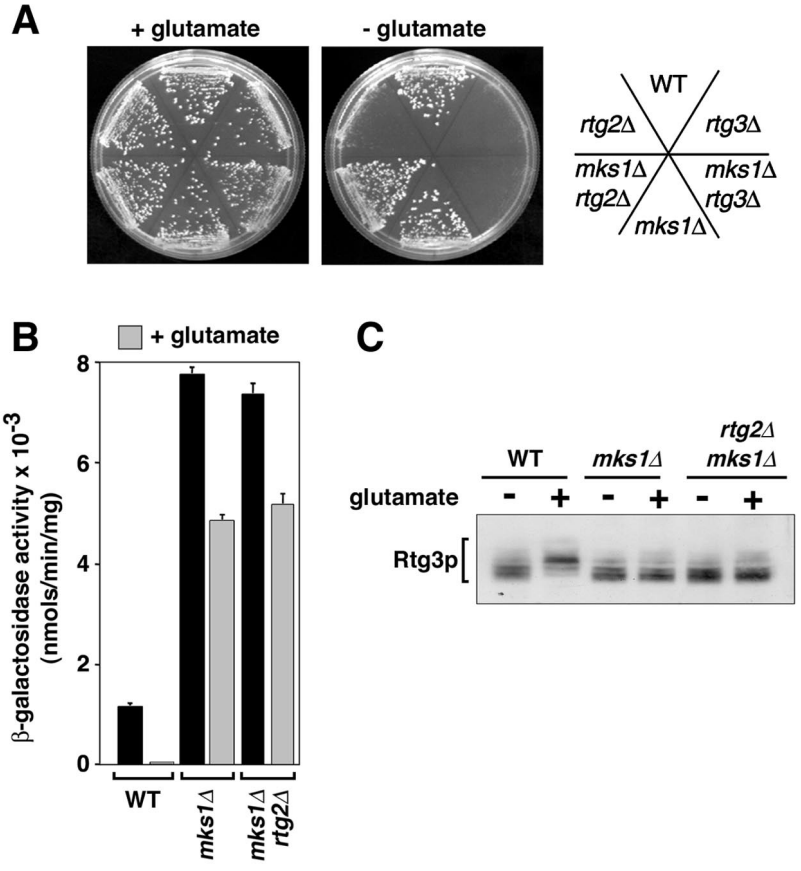

Figure 3. Inactivation of *MKS1* alters glutamate regulation of the *RTG* pathway. (A) Glutamate auxotrophy of *rtg2*Δ but not of *rtg3*Δ cells is reversed by the *mks1*Δ mutation. Derivatives of PSY142 cells were plated on YNBD medium without or with 0.01% glutamate. (B) *CIT2-lacZ* reporter gene expression is not significantly repressed by glutamate in *mks1*Δ cells. β-Galactosidase assays were carried out on whole-cell extracts of the indicated strains derived from PSY142 grown in YNBD medium with or without supplementation with 0.2% glutamate. (C) Glutamate-dependent phosphorylation of Rtg3p is reversed by the *mks1*Δ mutation. The increase in phosphorylation of Rtg3p is observed when wild-type PSY142 ρ^+ cells are grown in YNBD medium containing 0.2% glutamate but not in *mks1*Δ or *mks1*Δ *rtg2*Δ derivatives.

Mks1p Is in a Complex with Rtg2p

The dramatic decrease in the abundance of Mks1p$(HA)_3$ observed in *rtg2*Δ cells was intriguing. Northern blot analysis of *MKS1* mRNA showed that there was no difference in transcript levels between wild-type and *rtg2*Δ cells (Figure 6A). This raised the possibility that Mks1p might be present in a complex with Rtg2p and that in *rtg2*Δ cells, Mks1p would be destabilized. We therefore determined whether Mks1p and Rtg2p could be coprecipitated from whole-cell extracts of ρ^+ cells grown in rich raffinose medium. Extracts of cells coexpressing a C-terminal, triple myc tag of Mks1p [Mks1p$(myc)_3$] and an N-terminal triple HA-tagged derivative of Rtg2p [Rtg2p$(HA)_3$] were immunoprecipitated with anti-myc antibody and analyzed by Western blotting. These experiments show that the anti-myc antibody coprecipitated Rtg2p$(HA)_3$, and did so only in cells expressing

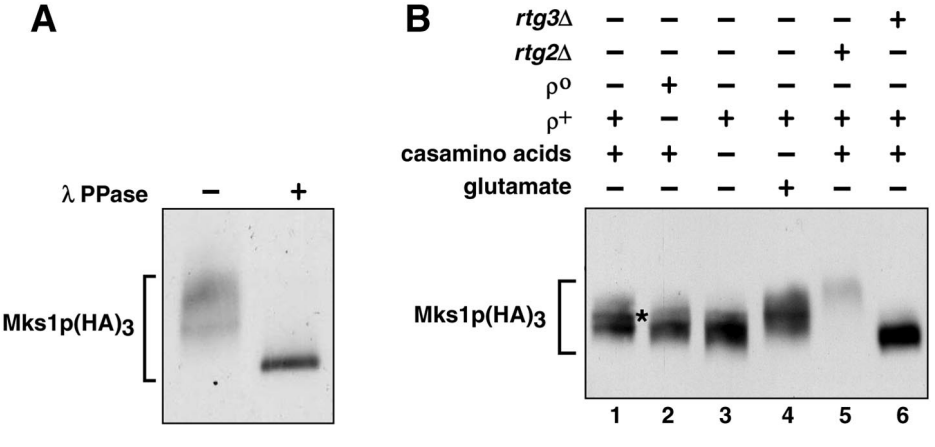

Figure 4. Mks1p is a phosphoprotein. (A) Mks1$(HA)_3$ was expressed in ρ^+ *mks1*Δ cells grown in YNBD medium containing 0.2% glutamate. Mks1p$(HA)_3$ was detected by Western blotting with a monoclonal anti-HA antibody either with or without pretreatment with λ phosphatase (λ PPase). (B) Western blot analysis of Mks1p$(HA)_3$ in extracts of ρ^+ or ρ^o cells grown in YNBD medium with or without the addition of 1% casamino acids or 0.2% glutamate as indicated. All strains were derived from PSY142. The asterisk indicates the position of a more phosphorylated species.

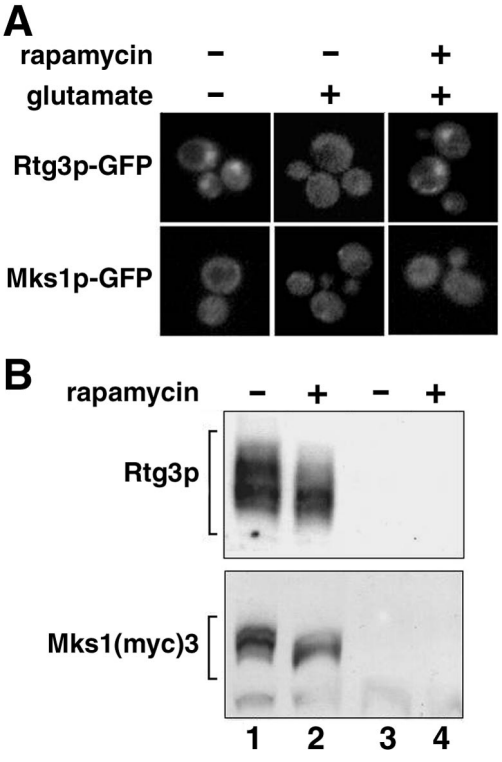

Figure 5. Effects of rapamycin on Rtg3p and Mks1p. (A) Effects of rapamycin and glutamate on the subcellular localization of Rtg3p-GFP and Mks1p-GFP. PSY142 ρ^+ cells expressing Rtg3p-GFP or Mks1p-GFP from constructs transplaced into the respective chromosomal *RTG3* or *MKS1* loci treated for 30 min with 200 ng/ml rapamycin (+) or vehicle alone (−) were grown in YNBD with or without the addition of 0.2% glutamate. (B) Rapamycin induces dephosphorylation of Rtg3p and Mks1p. Extracts from PSY142 ρ^+ cells grown in YNBD supplemented with 0.01% glutamate and treated with rapamycin or vehicle as described in A were analyzed by Western blotting with Rtg3p polyclonal anti-Rtg3p antiserum or monoclonal anti-myc antibody. Lanes 3 and 4 are extracts prepared from *rtg3*Δ cells (top) or from cells not containing the construct encoding Mks1(myc)$_3$ (bottom).

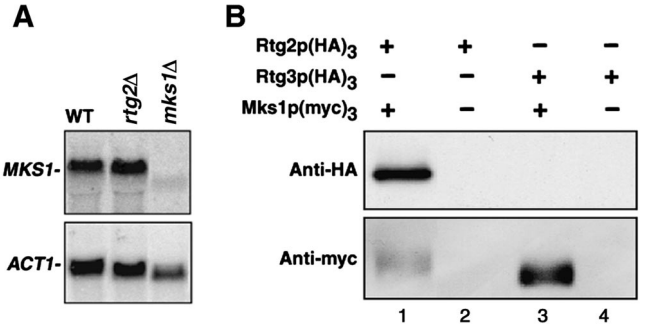

Figure 6. Coimmunoprecipitation of Mks1p and Rtg2p. (A) Northern blot analysis of *MKS1* transcripts were determined on total RNA prepared from PSY142 cells (B) Coimmunoprecipitation of (myc)$_3$- and (HA)$_3$-tagged derivatives of Mks1p and Rtg2p, respectively.

Mks1p(myc)$_3$ (Figure 6B, lanes 1 and 2); no detectable HA reactivity was observed in the immunoprecipitates of extracts from cells expressing a different HA-tagged protein, Rtg3p(HA)$_3$ (Figure 6B, lanes 3 and 4). In a reciprocal experiment in which extracts from cells expressing Rtg2p(myc)$_3$ and Mks1p(HA)$_3$ or Rtg3p(HA)$_3$ derivatives were immunoprecipitated with anti-myc antibody to pull-down Rtg2p, Mks1p(HA)$_3$, but not Rtg3p(HA)$_3$, was coprecipitated (our unpublished data). These data strongly suggest that Rtg2p and Mks1p are present in a complex that is not likely to include Rtg3p.

Inactivation of RTG Pathway Induces [URE3]

Recent studies have shown that Mks1p is required for the formation of [URE3], the inactive prion form of the negative regulator of Gln3, Ure2p (Edskes and Wickner, 2000). [URE3] was originally identified as a dominant, non-Mendelian determinant (Lacroute, 1971; Aigle and Lacroute, 1975), and first proposed by Wickner (1994) to be a yeast version of a prion. The infectious [URE3] prion is inherited as a stable, cytoplasmic element and shows altered structural properties based on the appearance of aggregates in vivo, protease resistance, and the ability to form amyloid in vitro (Taylor *et al.*, 1999; Wickner *et al.*, 2000). In the presence of high-quality nitrogen sources, the NCR pathway is inhibited by interaction between Ure2p and Gln3. Inactivation of Ure2p by its conversion to [URE3] releases Gln3p, allowing the expression of genes, such as *DAL5* encoding an allantoate permease, thus enabling cells to take up USA. Given the apparent dual role of Mks1p in the *RTG* pathway and in [URE3] formation, we asked whether the *RTG* pathway itself influences [URE3] formation. To this end, we determined the effect of *rtg2*Δ and *rtg3*Δ mutations on the appearance of USA$^+$ cells in two separate cultures of two randomly selected colonies of a derivative of strain MLY42 (Figure 7A). In the *rtg2*Δ and *rtg3*Δ strains, the frequency of USA$^+$ colonies increased dramatically 20–30-fold over the spontaneous level of USA$^+$ colonies in wild-type cells. Similar results were obtained with strain CC34 (our unpublished data). We verified that the USA$^+$ cells that arose in cultures of the *rtg2*Δ and *rtg3*Δ strains were [URE3]: first, when mated to a USA$^-$ tester strain, the resultant diploids were USA$^+$. Analysis of tetrads obtained after sporulation of the USA$^+$ diploids showed a non-Mendelian segregation pattern for the USA$^+$ phenotype, e.g., 4:0 and 0:4, in contrast to the strict 2:2 segregation of nuclear markers. Second, the USA$^+$ phenotype could be cured to USA$^-$ by growth of cells on YPD medium containing 5 mM guanidine HCl, consistent with a previous report on the curing of the [URE3] prion (Wickner, 1994). Finally, to exclude the possibility that the *rtg2* mutations result in some metabolic changes or inhibition of Ure2p function in some fraction of the cells that allow those cells to take up ureidosuccinic acid, and which is independent of [URE3] formation, we transformed a functional *RTG2* gene on a centromeric plasmid into *rtg2*Δ USA$^+$ cells. Despite restoration of the *RTG* pathway, all of the transformants remained USA$^+$ (our unpublished data). These findings, together with the properties of dominance, non-Mendelian meiotic segregation, and curing, confirm that the USA$^+$ colonies were [URE3].

In agreement with the findings from Wickner's laboratory (Edskes *et al.*, 1999; Edskes and Wickner, 2000), inactivation

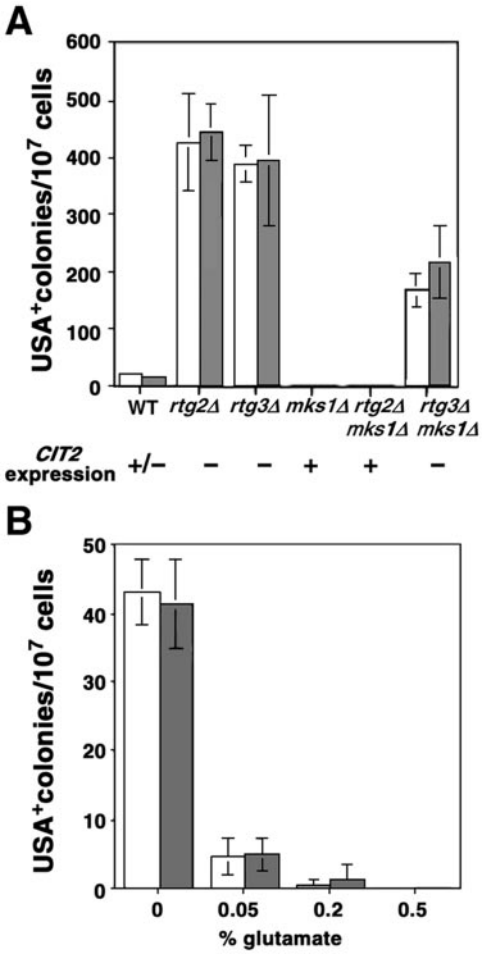

Figure 7. Effects of *rtg* mutations and glutamate on [URE3] formation. Two independent colonies of an MLY42 derivative (*MATα ura2*) were analyzed in parallel as indicated by the unshaded and shaded bars. (A) Effects of inactivation of *RTG* and *MKS1* on [URE3] formation. Wild-type (derived from MLY42, a Σ1278b background strain) and isogenic *rtg2*Δ, *rtg3*Δ, *mks1*Δ, *rtg2*Δ *mks1*Δ, and *mks1*Δ *rtg3*Δ mutant derivatives (all contain an *ura2*Δ*::kanMX4* mutation) were grown in YPD medium for 2 d to saturation. Cells were pelleted and washed twice with sterile water. For each strain, 10^6 or 10^7 cells were plated on each of seven YNBD plates supplemented with 200 μg/ml ureidosuccinic acid and 0.01% glutamate. The number of USA^+ colonies per plate was determined after 5 d of incubation at 30°C. Two independent colonies of each strain were analyzed as indicated by the shaded and unshaded bars. Error bars indicate SD of colony numbers from seven plates. *CIT2* expression refers to the qualitative level of *CIT2* expression in the various strains grown in YPD medium. (B) Glutamate inhibits [URE3] formation. Cells were cultured and plated in the same way as in A except that YNBD plates supplemented with 200 μg/ml ureidosuccinic acid and the indicated amounts of glutamate were used. Error bars indicate SD of colony numbers from seven plates.

of *MKS1* blocked the appearance of spontaneous USA^+ colonies (Figure 7A). In a similar manner, the *mks1*Δ mutation blocked the appearance of USA^+ colonies in the *rtg2*Δ mutants. In striking contrast, however, the *rtg3*Δ mutation reversed the block in USA^+ colony formation observed in *mks1*Δ cells, giving a level of USA^+ colonies about one-half of that observed in *rtg3*Δ cells alone. It is important to note, as is indicated in Figure 7A, that these effects of the *mks1*Δ mutation correlate directly with the presence or absence of *CIT2* expression in those mutant cells (Figure 1). These results suggest that the control of [URE3] formation by *MKS1* is effected at least in part through its control of *RTG* gene function. It has been reported that overproduction of Ure2p can lead to an increase in [URE3] formation (Wickner, 1994). Thus, one formal explanation for the increase in [URE3] formation when the retrograde pathway is inactivated is that it leads to an increased amount of Ure2p. However, using a functional GFP-tagged version of Ure2p and GFP-specific antiserum, we found that in *rtg2* mutant cells, there was no detectable increase in the amount of Ure2p in cells in which the *RTG* pathway was inactivated (our unpublished data).

Glutamate Represses [URE3] Formation

How does the *RTG* pathway regulate [URE3] formation? Because a primary function of *RTG* target gene expression is to maintain intracellular supplies of glutamate, we reasoned that glutamate itself might regulate [URE3] formation. To test this, we determined the frequency of USA^+ colonies that arose spontaneously in cultures of wild-type cells grown on minimal ammonia medium containing increasing concentrations of glutamate (Figure 7B). The results of this experiment show that the number of USA^+ colonies decreased with increasing concentrations of glutamate in the medium. At 0.5% glutamate, USA^+ colony formation was essentially completely inhibited. In control experiments, we have determined that glutamate has no effect on the survival of USA^+ cells, does not convert USA^+ to USA^- cells, i.e., does not cure the [URE3] prion, and that the USA^+ [URE3] cells can grow on USA medium containing 0.2% glutamate (our unpublished data). Similar observations for growth of [URE3] cells were noted by Aigle and Lacroute (1975). Together, these data indicate that glutamate is a negative regulator of [URE3] formation.

DISCUSSION

The findings presented herein show that *MKS1* is a negative regulator of the *RTG* pathway. We identified *MKS1* in a screen for mutations that would suppress the block in *RTG*-dependent gene expression in *rtg2*Δ cells. When *MKS1* is inactivated, *RTG*-dependent gene expression is constitutive and no longer requires Rtg2p. Previous studies have established that Rtg2p is a proximal sensor of mitochondrial dysfunction, acting upstream of the transcription factors Rtg1p and Rtg3p (Rothermel *et al.*, 1997; Liu and Butow, 1999; Sekito *et al.*, 2000). Because the suppression of the block in gene expression in *rtg2*Δ cells by an *mks1* null mutation still requires Rtg3p (as well as Rtg1p), the suppression is not likely to involve the activation of some alternative pathway of gene expression, for example, by the recruitment of surrogate transcription factors to the *CIT2* promoter.

A number of well-defined physiological and molecular signatures are associated with regulation of the *RTG* pathway (Liao and Butow, 1993; Liu and Butow, 1999; Sekito *et al.*, 2000). These include 1) glutamate auxotrophy of *rtg* mutant cells with compromised or reduced mitochondrial

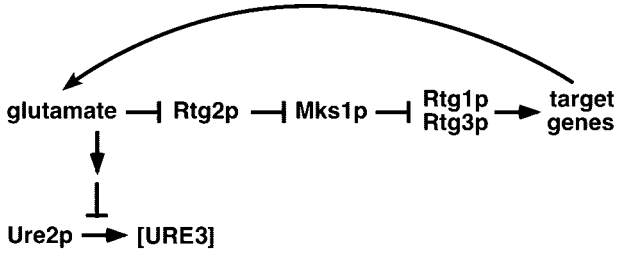

Figure 8. Model linking the negative feedback control of glutamate levels by the *RTG* pathway and [URE3] formation. Glutamate (or glutamine) could inhibit [URE3] formation directly or through an intermediary sensing mechanism.

function, 2) translocation of Rtg1p and Rtg3p from the cytoplasm to the nucleus when *RTG* target gene expression is activated, 3) partial dephosphorylation of Rtg3p that accompanies its nuclear accumulation, and 4) hyperphosphorylation and cytoplasmic retention of Rtg3p in *rtg2*Δ cells. Inactivation of *MKS1* in wild-type ρ^+ and in *rtg2*Δ cells affects all of the above-mentioned processes in a manner fully consistent with the high-level of *CIT2* expression observed in those mutants. Together, these findings suggest that Mks1p acts between Rtg2p and the Rtg1p-Rtg3p transcription complex in its negative regulation of *RTG*-dependent gene expression (Figure 8). A recent report has also suggested that Mks1p regulates Rtg1p-Rtg3p activity (Shamji *et al.*, 2000). However, in contrast to the conclusions drawn from our findings, Mks1p was proposed to be a positive rather than a negative regulator of the *RTG* pathway. That conclusion was based on the observation that *RTG* target gene expression is not responsive to rapamycin in *mks1*Δ cells. In the experiments of Shamji *et al.* (2000), it is likely that the level of expression of the *RTG* target genes examined was already at or close to maximum because of the *mks1*Δ mutation, as we have described herein, and thus expression of those genes would have appeared to have been unresponsive to rapamycin.

Our studies have revealed for the first time that Mks1p is a phosphoprotein located in the cytoplasm whose phosphorylation state is regulated. It is intriguing that the phosphorylation state of Mks1p responds to the same signals and in the same way as Rtg3p phosphorylation. One obvious implication of these findings is that the phosphorylation state of Mks1p affects its regulation of the *RTG* pathway. Although we found that Mks1p is dephosphorylated in *rtg3*Δ cells in which the *RTG* pathway is inactive, that result can be easily reconciled with the notion that dephosphorylation of Mks1p relieves its negative regulation of *RTG*-dependent gene expression: we previously demonstrated that there is a feedback response in cells lacking a functional Rtg3p, as if these cells were attempting to activate the *RTG* pathway (Sekito *et al.*, 2000). In those experiments, cells containing a cytoplasmic derivative of Rtg3p (ΔNLS) that was unable to translocate into the nucleus because it lacked its nuclear localization sequence, Rtg3p was dephosphorylated. When those cells were also transformed with a construct expressing a functional copy of Rtg3p, the ΔNLS Rtg3p derivative, still cytoplasmic, became phosphorylated. Ongoing studies directed at a mutational analysis of potential phosphorylation sites in Mks1p should clarify the relation between phosphorylation of the protein and its regulation of the *RTG* pathway.

Mks1p has been proposed to act within a number of different pathways in the cell, including the Ras-cAMP pathway (Matsuura and Anraku, 1993), lysine biosynthesis (Feller *et al.*, 1997), the TOR kinase pathway (Shamji *et al.*, 2000), and as a regulator of Ure2p (Edskes *et al.*, 1999; Edskes and Wickner, 2000). Our finding that Mks1p is in a complex with Rtg2p implies an interplay between these two proteins in their regulation of *RTG*-dependent gene expression. The biochemical function of neither of these proteins is known, so that it is not possible to conclude at this time how their colocalization in a complex might regulate *RTG*-dependent gene expression. However, if we assume that the phosphorylation state of Mks1p is important for its regulation of the retrograde pathway, phosphorylation is not likely to be involved in the entry or release of either of these components into a common complex, because Mks1p and Rtg2p can be coimmunoprecipitated independently of the observed changes in Mks1p phosphorylation (Sekito, unpublished observations).

One possible clue to a function for Mks1p is that there are two regions of the protein, which together, share ~50% sequence similarity to a 234-amino acid stretch in the N-terminal region of the Ppz1p phosphatase of yeast. The N-terminal domain of Ppz1p has been proposed to have a regulatory function for its catalytic phosphatase domain located in the C-terminal part of protein (Hughes *et al.*, 1993). This observation, together with the finding that Mks1p is present in a complex with Rtg2p, may offer some clues as to how Mks1p might regulate the *RTG* pathway. As noted above, Rtg2p functions as a proximal sensor of mitochondrial dysfunction, translating mitochondrial signals to the Rtg1p–Rtg3p complex. Rtg2p thus acts as a switch to keep Rtg1p and Rtg3p tethered in the cytoplasm when the retrograde pathway is off, and to enable their release for nuclear entry when the pathway is turned on. This Rtg2p switch could be effected by modulating, via Mks1p, a phosphatase activity acting upon Rtg3p, so as to initiate Rtg3p's translocation to the nucleus. In the absence of Mks1p, the putative phosphatase would be constitutively active, resulting in *RTG* target gene expression that is no longer dependent on Rtg2p.

Recent genome-wide transcription studies and analyses of changes in gene expression resulting from dysfunctional mitochondria, treatment of cells with rapamycin, or growth of cells on different nitrogen sources show that both carbon and nitrogen metabolism are connected via the *RTG* pathway (Komeili *et al.*, 2000; Shamji *et al.*, 2000; Epstein *et al.*, 2001). Our experiments are in agreement with previous studies (Komeili *et al.*, 2000; Shamji *et al.*, 2000) showing that treatment of cells with rapamycin activates *RTG*-dependent gene expression. We have found, however, that rapamycin treatment, as well as all other regimes we have tested that activate *RTG*-dependent gene expression, results in a dephosphorylation of Rtg3p, rather than an increase in the phosphorylation of the protein as reported by Komeili *et al.* (2000). Presently, we have no explanation for this discrepancy. It is worth noting that mutation of a number of potential phosphorylation sites in Rtg3p results in its constitutive nuclear localization (Sekito, unpublished observations), con-

sistent with the view that dephosphorylation of Rtg3p is associated with its nuclear accumulation.

A striking connection between the *RTG* pathway and nitrogen metabolism was revealed by the finding that inactivation of the *RTG*-pathway by the *rtg2*Δ or *rtg3*Δ mutations results in a dramatic increase in the frequency of [URE3] cells. This increase in [URE3] formation can occur independently of *MKS1* and is clearly related to a loss of *RTG* target gene expression. Thus, in *rtg2*Δ *mks1*Δ cells, in which *CIT2* expression is high, [URE3] formation is at background levels, whereas in an *rtg3*Δ *mks1*Δ mutant, in which *CIT2* expression is blocked, [URE3] formation is significantly higher than in wild-type cells. Our result showing that glutamate is a negative regulator of [URE3] formation explains how the *rtg2*Δ or *rtg3*Δ mutations would increase [URE3] formation and how the *mks1*Δ mutation alone decreases it (Edskes and Wickner, 2000): inactivation of the *RTG* pathway starves cells for glutamate (indeed, such cells are glutamate auxotrophs) (Liao and Butow, 1993; Jia *et al.*, 1997). The low levels of glutamate would result in an increase in frequency of [URE3] formation; in contrast, high levels of *RTG* gene activity due to the inactivation of *MKS1* would lead to increased glutamate levels and a suppression of [URE3] formation. As summarized in the model of Figure 8, the targets of the *RTG* genes that are directly involved in glutamate synthesis, together with the negative feedback control of the *RTG* pathway by glutamate, would influence the formation of the [URE3] prion. Our results do not rule out the possibility, however, that Mks1p might also regulate [URE3] formation by direct interaction with Ure2p, as suggested by Edskes and Wickner (2000), especially given the result shown in Figure 7A that the dramatic increase in [URE3] formation in *rtg3*Δ cells is somewhat suppressed by the *mks1*Δ mutation.

What might be the logic behind the link between the *RTG* pathway and [URE3] formation? As noted above, when cells are faced with poor nitrogen sources, both the NCR pathway and *RTG*-dependent gene expression are activated (Komeili *et al.*, 2000; Shamji *et al.*, 2000). The NCR pathway is activated because Ure2p has been released from a complex with Gln3p, allowing Gln3p to translocate to the nucleus (Beck and Hall, 1999). Because Ure2p needs to remain in an "active" state, poised to reassociate with and inactivate Gln3p when a high-quality nitrogen source becomes available, it would be a clear advantage to the cell to suppress conversion of Ure2p to the inactive [URE3] prion form, so as allow cells to switch rapidly between states suitable for the utilization of different quality nitrogen sources. As we have shown herein, such suppression occurs when *RTG*-dependent gene activity is high.

Finally, our results also shed light on how *MKS1* could behave as a negative regulator of lysine biosynthesis (Feller *et al.*, 1997). α-Ketoglutarate is a precursor to lysine, so that down-regulation of α-ketoglutarate synthesis would also down-regulate the lysine biosynthetic pathway. Because *MKS1* negatively regulates *RTG* gene function, which is required for the production of α-ketoglutarate (Liu and Butow, 1999), a strong down-regulation of the *RTG* pathway by *MKS1* would also be manifest as a down-regulation of lysine biosynthesis. Altogether, these findings underscore the central role played by the *RTG* pathway in connecting nitrogen and carbon metabolism to the functional state of mitochondria.

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REFERENCES

Aigle, M., and Lacroute, F. (1975). Genetical aspects of [URE3], a non mitochondrial, cytoplasmically inherited mutation in yeast. Mol. Gen. Genet. *136*, 327–335.

Amuthan, G., Biswas, G., Zhang, S.Y., Klein-Szanto, A., Vijayasarathy, C., and Avadhani, N.G. (2001). Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. EMBO J. *20*, 1910–1920.

Beck, T., and Hall, M.N. (1999). The TOR signaling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature *402*, 689–692.

Biswas, G., Adebanjo, O.A., Freedman, B.D., Anandatheerthavarada, H.K., Vijayasarathy, C., Zaidi, M., Kotlikoff, M., and Avadhani, N.G. (1999). Retrograde Ca2+ signaling in C2C12 skeletal myocytes in response to mitochondrial genetic and metabolic stress: a novel mode of inter-organelle crosstalk. EMBO J. *18*, 522–533.

Blinder, D., Coschigano, P.W., and Magasanik, B. (1996). Interaction of the GATA factor Gln3p with the nitrogen regulator Ure2p in *Saccharomyces cerevisiae*. J. Bacteriol. *178*, 4734–4746.

Bork, P., Sander, C., and Valencia, A. (1992). An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin and hsp70 heat shock proteins. Proc. Natl. Acad. Sci. USA *89*, 7290–7294.

Coschigano, P.W., and Magasanik, B. (1991). The URE2 gene product of *Saccharomyces cerevisiae* plays an important role in the cellular response to the nitrogen source and has homology to glutathione s-transferases. Mol. Cell. Biol. *11*, 822–832.

Courchesne, W.E., and Magasanik, B. (1988). Regulation of nitrogen assimilation in *Saccharomyces cerevisiae*: roles of the URE2 and GLN3 genes. J. Bacteriol. *170*, 708–713.

Edskes, H.K., Hanover, J.A., and Wickner, R.B. (1999). Mks1p is a regulator of nitrogen catabolism upstream of Ure2p in *Saccharomyces cerevisiae*. Genetics *153*, 585–594.

Edskes, H.K., and Wickner, R.B. (2000). A protein required for prion generation: [URE3] induction requires the Ras-regulated Mks1 protein. Proc. Natl. Acad. Sci. USA *97*, 6625–6629.

Epstein, C.B., Waddle, J.A., Hale IV, W., Davé, V., Thornton, J., Macatee, T.L., Garner, H.R., and Butow, H.R. (2001). Genome-wide responses to mitochondrial dysfunctions. Mol. Biol. Cell *12*, 297–308.

Feller, A., Ramos, F., Pierard, A., and Dubois, E. (1997). Lys80p of *Saccharomyces cerevisiae*, previously proposed as a specific repressor of LYS genes, is a pleiotropic regulatory factor identical to Mks1p. Yeast *13*, 1337–1346.

Forsburg, S.L., and Guarente, L. (1989). Communication between mitochondria and the nucleus in regulation of cytochrome genes in the yeast *Saccharomyces cerevisiae*. Annu. Rev. Cell Biol. *5*, 153–180.

Hallstrom, T.C., and Moye-Rowley, S. (2000). Multiple signals from dysfunctional mitochondria activate the pleiotropic drug resistance pathway in *Saccharomyces cerevisiae*. J. Biol. Chem. *275*, 34347–37356.

Heddi, A., Stepien, G., Benke, P.J., and Wallace, D.C. (1999). Coordinate induction of energy gene expression in tissues of mitochondrial disease patients. J. Biol. Chem. *274*, 22968–22976.

Hughes, V., Mulle, A., Stark, M.J.R., and Cohen, P.T.W. (1993). Both isoforms of protein phosphatase Z are essential for the maintenance of cell size and integrity in *Saccharomyces cerevisiae* in response to osmotic stress. Eur. J. Biochem. *216*, 269–279.

Jia, Y., Rothermel, B., Thornton, J., and Butow, R.A. (1997). A basic helix-loop-helix zipper transcription complex functions in a signaling pathway from mitochondria to the nucleus. Mol. Cell. Biol. *17*, 1110–1117.

Komeili, A., Wedaman, K.P., O'Shea, E.K., and Powers, T. (2000). Mechanism of metabolic control: target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. J. Cell Biol. *151*, 863–878.

Lacroute, F. (1971). Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast. J. Bacteriol. *106*, 519–522.

Liao, X., and Butow, R.A. (1993). *RTG1* and *RTG2*: Two yeast genes required for a novel path of communication from mitochondria to the nucleus. Cell *72*, 61–71.

Liao, X.S., Small, W.C., Srere, P.A., and Butow, R.A. (1991). Intramitochondrial functions regulate nonmitochondrial citrate synthase (CIT2) expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *11*, 38–46.

Liu, Z., and Butow, R.A. (1999). A transcriptional switch in the expression of yeast tricarboxylic acid cycle genes in response to a reduction or loss of respiratory function. Mol. Cell. Biol. *19*, 6720–6728.

Lunardi, J., and Attardi, G. (1991). Differential regulation of expression of the multiple ADP/ATP translocase genes in human cells. J. Biol. Chem. *266*, 16534–16540.

Matsuura, A., and Anraku, Y. (1993). Characterization of the MKS1 gene, a new negative regulator of the Ras-cyclic AMP pathway in *Saccharomyces cerevisiae*. Mol. Gen. Genet. *238*, 6–16.

Mitchell, A.P., and Magasanik, B. (1984). Regulation of glutamine-repressible gene products by the GLN3 function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *4*, 2758–2766.

Murdock, D.G., Boone, B.E., Esposito, L.A., and Wallace, D.C. (1999). Up-regulation of nuclear and mitochondrial genes in the skeletal muscle of mice lacking the heart/muscle isoform of the adenine nucleotide translocator. J. Biol. Chem. *274*, 14429–14433.

Parikh, V.S., Conrad-Webb, H., Docherty, R., and Butow, R.A. (1989). Interaction between the yeast mitochondrial and nuclear genomes influences the abundance of novel transcripts derived from the spacer region of the nuclear ribosomal DNA repeat. Mol. Cell. Biol. *9*, 1897–1907.

Parikh, V.S., Morgan, M.M., Scott, R., Clements, L.S., and Butow, R.A. (1987). The mitochondrial genotype can influence nuclear gene expression in yeast. Science *235*, 576–580.

Rose, M.D., Winston, F., and Heiter, P. (1990). Methods in Yeast Genetics: A Laboratory Course Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Rosenkrantz, M., Kell, C.S., Pennell, E.A., and Devenish, L.J. (1994). The HAP2,3,4 transcriptional activator is required for derepression of the yeast citrate synthase gene, *CIT1*. Mol. Microbiol. *13*, 119–131.

Rothermel, B., Thornton, J., and Butow, R.A. (1997). Rtg3p, a basic helix-loop-helix/leucine zipper protein functions in mitochondrial-induced changes in gene expression, contains independent activation domains. J. Biol. Chem. *272*, 19801–19807.

Scarpulla, R.C. (1997). Nuclear control of respiratory chain expression in mammalian cells. J. Bioenerg. Biomembr. *29*, 109–119.

Sekito, T., Thornton, J., and Butow, R.A. (2000). Mitochondria-to-nuclear signaling is regulated by the subcellular localization of the transcription factors Rtg1p and Rtg3p. Mol. Biol. Cell *11*, 2103–2115.

Shamji, A.F., Kuruvilla, F.G., and Schreiber, S.L. (2000). Partitioning the transcriptional program induced by rapamycin among the effectors of the TOR proteins. Curr. Biol. *10*, 1574–1581.

Taylor, K.L., Cheng, N., Williams, R.W., Steven, A.C., and Wickner, R.B. (1999). Prion domain initiation of amyloid formation in vitro from native Ure2p. Science *283*, 1339–1343.

Traven, A., Wong, J.M., Xu, D., Sopta, M., and Ingles, C.J. (2000). Inter-organellar communication: altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. J. Biol. Chem. *27*, 27.

Wach, A., Brachat, A., Pohlmann, R., and Philippsen, P. (1994). New heterologous modules for classical PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast *10*, 1793–1808.

Wickner, R.B. (1994). [URE3] as an altered *URE2* protein: evidence for a prion analog in *Saccharomyces cerevisiae*. Science *264*, 566–569.

Wickner, R.B., Taylor, K.L., Edskes, H.K., Maddelein, M.L., Moriyama, H., and Roberts, B.T. (2000). Prions of yeast as heritable amyloidoses. J. Struct. Biol. *130*, 310–322.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C., and Spiegelman, B.M. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell *98*, 115–124.