Sls1p Is a Membrane-Bound Regulator of Transcription-Coupled Processes Involved in *Saccharomyces cerevisiae* Mitochondrial Gene Expression

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ABSTRACT

Mitochondrial translation is largely membrane-associated in *S. cerevisiae*. Recently, we discovered that the matrix protein Nam1p binds the amino-terminal domain of yeast mtRNA polymerase to couple translation and/or RNA-processing events to transcription. To gain additional insight into these transcription-coupled processes, we performed a genetic screen for genes that suppress the petite phenotype of a point mutation in mtRNA polymerase (*rpo41-R129D*) when overexpressed. One suppressor identified in this screen was *SLS1*, which encodes a mitochondrial membrane protein required for assembly of respiratory-chain enzyme complexes III and IV. The mtRNA-processing defects associated with the *rpo41-R129D* mutation were corrected in the suppressed strain, linking Sls1p to a pathway that includes mtRNA polymerase and Nam1p. This was supported by the observation that *SLS1* overexpression rescued the petite phenotype of a *NAM1* null mutation. In contrast, overexpression of Nam1p did not rescue the petite phenotype of a *SLS1* null mutation, indicating that Nam1p and Sls1p are not functionally redundant but rather exist in an ordered pathway. On the basis of these data, a model in which Nam1p coordinates the delivery of newly synthesized transcripts to the membrane, where Sls1p directs or regulates their subsequent handling by membrane-bound factors involved in translation, is proposed.

In most eukaryotic cells, genetic information is housed in both the nucleus and mitochondria. The mitochondrial genome (mitochondrial DNA) encodes an essential subset of the protein components of the mitochondrial oxidative phosphorylation system, or respiratory chain, that comprises up to five multi-protein enzymatic complexes that are located in the inner mitochondrial membrane (Shadel 1999). The remainder of the protein components in these complexes is encoded in the nucleus. Therefore, mutations in mitochondrial DNA (mtDNA) or nuclear genes encoding these proteins can result in loss of mitochondrial respiration capacity and decreased cellular ATP production. In humans, mutations of this type cause specific diseases and likely contribute to late-onset neurodegenerative disorders and aging (Wallace 1999; Smeitink *et al.* 2001). Currently, our knowledge of mitochondrial genetics and disease is incomplete due to the lack of a complete understanding of fundamental aspects of mitochondrial gene expression and mtDNA maintenance (Shadel and Clayton 1997).

In *Saccharomyces cerevisiae*, expression of mtDNA-encoded genes is initiated by a dedicated mtRNA polymerase, encoded by the nuclear *RPO41* gene (Greenleaf *et al.* 1986). Recently, we have shown that this protein consists of at least two functional regions, a large C-terminal region (~100 kD), comprising eight motifs with strong similarity to bacteriophage RNA polymerases (Masters *et al.* 1987), and an N-terminal extension (~40 kD) that harbors a functional domain that is largely dispensable for transcription initiation, but required for mitochondrial genome stability (Wang and Shadel 1999). One function of this domain is to bind Nam1p, a mitochondrial matrix protein involved in translation and RNA-processing events, suggesting that these post-transcriptional events are coupled to transcription (Rodeheffer *et al.* 2001). While the precise function of Nam1p in these processes remains to be determined, others have speculated that it functions as a chaperone for mitochondrial transcripts in the matrix (Wallis *et al.* 1994; Manthey *et al.* 1998).

The latter stages of mitochondrial gene expression are complex and involve a number of sequential events that are likely coordinated with each other. For example, due to the polycistronic nature of most mitochondrial transcripts, a large number of RNA-processing events are required to liberate the mature mRNAs, tRNAs, and rRNAs for translation (Shadel and Clayton 1997). In yeast, this includes tRNA excision, a variety of mRNA cleavages, and the removal of introns from certain mRNAs (Attardi and Schatz 1988; Dieckmann and Staples 1994). The latter of these events is complicated further because translation is necessary for intron removal due to the presence of intron-encoded maturases that are required for splicing (Pel and Gri-

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vell 1993). Finally, translation of mitochondrial messages requires gene-specific translational activator proteins that bind to the 5′-untranslated regions (5′-UTR) of mRNAs to facilitate translation (Costanzo and Fox 1990), presumably by promoting ribosome association (McMullin *et al.* 1990). Many of these translational activators are associated with the inner membrane (McMullin and Fox 1993; Wiesenberger and Fox 1997; Manthey *et al.* 1998; Green-Willms *et al.* 2001), indicating that mitochondrial translation is largely a membrane-associated process (Fox 1996). This situation, in principle, requires a mechanism to deliver newly synthesized transcripts to this location. Here, we present the results of a genetic study that provide evidence that an RNA-handling pathway exists in yeast mitochondria to coordinate transcription in the matrix to translation at the membrane.

MATERIALS AND METHODS

Plasmids: Most of the plasmids used in this study were derivatives of the yeast/*Escherichia coli* shuttle vectors pRS314 (*CEN/ARS, TRP1*) and pRS316 (*CEN/ARS, URA3*; Sikorski and Hieter 1989). The plasmids pRS314-*NAM1* and pRS316-*NAM1* contain a ~1.7-kb *Xba*I-*Bam*HI fragment spanning the *NAM1* gene inserted into pRS314 and pRS316, respectively. The plasmids pRS314-*SLS1* and pRS316-*SLS1* consist of a ~2.2-kb fragment spanning the *SLS1* gene inserted into pRS314 and pRS316, respectively. The plasmid pYES/GS-*NAM1* was obtained from Invitrogen (Carlsbad, CA; Genestorm clone yDL-044cy). The *NAM1* allele in this plasmid, which is tagged on its C terminus with a V5 epitope and under control of a galactose-inducible promoter, was excised on a *Sna*BI-*Xba*I fragment and ligated into the *Sma*I and *Spe*I sites in pRS314 to create the plasmid pRS314-*NAM1*t. The epitope-tagged version of Nam1p encoded by this plasmid is functional (Rodeheffer *et al.* 2001) and can complement the *NAM1* null allele in GS140 (data not shown).

The library plasmid pRMS5-6 responsible for suppression of the *rpo41-R129D* mutation in strain RMS5-6 is a λYES-R vector (Elledge *et al.* 1991) that contains a 5.1-kb yeast genomic insert from chromosome XII spanning three intact open reading frames (ORFs; Figure 1). Three plasmids that contain subclones of this original genomic insert (Figure 1) were constructed as follows: pRMS5-6Δ1, a ~3-kb *Xho*I fragment containing intact *SLS1* and yLR140w ORFs, was ligated into pRS316; pRMS5-6Δ2, a ~2.2-kb *Xho*I-*Spe*I fragment containing only an intact *SLS1* ORF, was ligated into pRS316; and pRMS5-6Δ3, a ~2-kb *Spe*I-*Dra*I fragment containing an intact *RRN5* ORF, was ligated into YEp352 (*URA3*, 2μ).

Yeast strains, growth media, and phenotypic selection: Yeast were grown in standard synthetic dextrose (SD) medium with nutritional supplements or YPG (glycerol-containing) medium as described (Sherman 1991). Where indicated, galactose (0.7%) was added to YPG medium (YPG-GAL). Assessment of mitochondrial petite phenotypes and plasmid shuffling were performed as described (Rodeheffer *et al.* 2001).

All yeast strains used in this study (Table 1) are derivatives of DBY2006 (α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2*). Construction of yeast strain GS122 has been described (Wang and Shadel 1999). GS129 is analogous to GS122 except the *RPO41*-containing *TRP1* plasmid remaining after plasmid shuffle contains the *rpo41-R129D* mutated allele instead of a wild-type allele (Rodeheffer *et al.* 2001). To construct the *NAM1* plasmid-shuffle strain (GS140), the *NAM1* chromosomal locus was disrupted with a *HIS3* cassette that was inserted at the *Sna*BI and *Xba*I restriction sites, located ~180 bp upstream of the Nam1p start codon and within the *NAM1* ORF, respectively. This chromosomal *NAM1* deletion/insertion in this strain was covered by a plasmid-borne copy of the *NAM1* gene under control of its own promoter (pRS316-*NAM1*). The *SLS1* plasmid-shuffle strain CMW2 is analogous to GS140, except the plasmid pRS316-*SLS1* covers the chromosomal disruption/insertion of the *SLS1* gene, which is a precise replacement of the *SLS1* ORF with a KanMX4 cassette. The yeast strains GS141 and GS142 were made by plasmid shuffle of GS140 after transformation with pRS314-*NAM1* and pRS314, respectively. Likewise, the yeast strains CMW3, CMW4, and CMW5 were made by plasmid shuffle of CMW2 after transformation with pRS314, pRS314-*SLS1*, and pRS314-*NAM1t*, respectively.

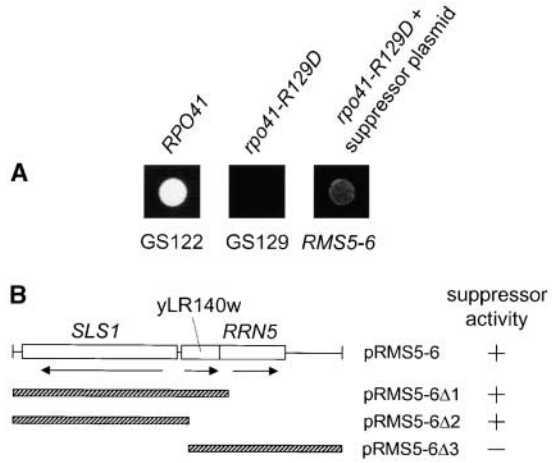

Figure 1.—Overexpression of *SLS1* suppresses the petite phenotype of the *rpo41-R129D* point mutation in mtRNA polymerase. (A) Mitochondrial petite phenotypes of the following strains (indicated at the bottom) were assessed at 37°: GS122, *RPO41* wild type; GS129, *rpo41-R129D*; and RMS5-6, a strain isolated in our genetic screen and found to contain a plasmid-borne suppressor of the *rpo41-R129D* mutation. Shown is the growth from a small volume of liquid culture of the indicated strain that was spotted onto solid YPG medium as described (Rodeheffer *et al.* 2001). The relevant genotype for each strain is labeled at the top. (B) Diagram of the genomic DNA insert in the pRMS5-6 suppressor plasmid from the strain RMS5-6 and deletion constructs used to delineate the gene responsible for suppressor activity. The 5.1-kb insert in pRMS5-6 (diagrammed at the top with the 5′–3′ orientation of the ORFs indicated with an arrow) contains three intact open reading frames (open boxes): *SLS1*, yLR140w, and *RRN5*. The portion of the original genomic insert retained (hatched boxes) in three plasmid derivatives of pRMS5-6 is diagrammed below (pRMS5-6Δ1 retains *SLS1* and yLR140w; pRMS5-6Δ2 retains intact *SLS1* only; and pRMS5-6Δ3 retains intact RRN5 only). The ability of each of these plasmids to suppress the *rpo41-R129D* mutation is indicated with a plus (+) or a minus (−) at the right.

Screening for suppressors of the *rpo41-R129D* mutation: The *S. cerevisiae* genomic library used in this study is contained in the plasmid λYES-R (Elledge *et al.* 1991) and was obtained from American Type Culture Collection (Manassas, VA). In our selection scheme, genes are overexpressed from this plas-

TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype	Reference
GS122	α*his3-Δ200 leu2-3,-112 ura3-52 trp-1-Δ1 ade2 rpo41Δ1::HIS3* + [pRS314-*RPO41*]	Wang and Shadel (1999)
GS124	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 rpo41Δ1::HIS3* + [pRS314-*rpo41Δ2*]	Wang and Shadel (1999)
GS125	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 rpo41Δ1::HIS3* + [pRS314-*rpo41Δ3*]	Wang and Shadel (1999)
GS128	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 rpo41Δ1::HIS3* + [pRS314-*rpo41-E119A/C121A*]	Rodeheffer *et al.* (2001)
GS129	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 rpo41Δ1::HIS3* + [pRS314-*rpo41-R129D*]	Rodeheffer *et al.* (2001)
GS130	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 rpo41Δ1::HIS3* + [pRS314-*rpo41-N152A/Y154A*]	Rodeheffer *et al.* (2001)
GS140	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 nam1Δ::HIS3* + [pRS316-*NAM1*]	This study
GS141	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 nam1Δ::HIS3* + [pRS314-*NAM1*]	This study
GS142	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 nam1Δ::HIS3* + [pRS314]	This study
ACB1	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 nam1Δ::HIS3* + [pRS314] + [pRS316-*SLS1*]	This study
CMW2	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 sls1Δ*::KanMX4 + [pRS316-*SLS1*]	This study
CMW3	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 sls1Δ*::KanMX4 + [pRS314]	This study
CMW4	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 sls1Δ*::KanMX4 + [pRS314-*SLS1*]	This study
CMW5	α*his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2 sls1Δ*::KanMX4 + [pRS314-*NAM1*t]	This study

mid because of gene dosage effects (*i.e.*, the plasmid is maintained at a copy number of four to five copies/cell) or by virtue of being inserted downstream of the resident *GAL* promoter in a manner that allows elevated transcription of the gene (in YPG medium, this promoter is active due to the absence of glucose repression). The yeast strain GS129 was transformed with the λYES-R library and Ura^+ transformants were selected at 30° on solid SD medium supplemented with leucine (30 mg/liter) and adenine (20 mg/liter). These strains were subsequently replica plated onto YPG medium and grown at 35° for several days to select for respiration competence. Putative suppressor strains were named with the prefix RMS (*R*NA polymerase *m*utant *s*uppressor). One strong suppressor strain isolated in this manner, RMS5-6, is characterized in this study. The library plasmid was isolated from RMS5-6 and transformed into a fresh GS129 strain to ensure that the suppressor phenotype was plasmid linked. This plasmid (pRMS5-6) was sequenced and this information was used to delineate the yeast genomic DNA insert in the suppressor plasmid as described (Wang and Shadel 1999).

Northern and immunoblot analyses: Isolation of total yeast mtRNA and detection of mature *COX1*, *COB*, and *COX3* messages was performed as described previously (Rodeheffer *et al.* 2001). Western immunoblot analysis of total cellular protein from the indicated yeast strains was performed as described (Wang and Shadel 1999), except the blot was probed with a rabbit polyclonal antibody raised against recombinant Nam1p (a gift from Dr. David A. Clayton, Stanford University).

RESULTS

Identification of *SLS1* as a suppressor of the mtRNA polymerase amino-terminal domain mutation *rpo41-R129D*: In a previous study (Rodeheffer *et al.* 2001), we characterized mutations in the amino-terminal domain of yeast mtRNA polymerase (Rpo41p) and revealed that this domain is involved in binding Nam1p in order to couple subsequent events involved in mitochondrial gene expression to the transcription machinery. One mutation described in that study (*rpo41-R129D*) resulted in a respiration-competent, but slow-growth, phenotype on glycerol medium (YPG) at 30° and a petite phenotype at higher temperatures. In the present study, we screened a plasmid library of yeast genomic DNA for genes that suppress the mitochondrial petite phenotype of the *rpo41-R129D* mutation at 35°. A total of 6 strains (of ~80,000 screened) that exhibited strong and reproducible suppression of the YPG growth defect of the *rpo41-R129D* mutant strain (GS129) were isolated. The plasmids that confer suppression activity to these strains were isolated and the genomic DNA fragment in each was identified. Three of these plasmids contained the yeast *MAT*α locus and are currently under investigation, two contained a portion of the nuclear rDNA repeat and are the subject of another manuscript, and the final plasmid (designated pRMS5-9) was found to contain a 5.1-kb genomic fragment that contained three yeast open reading frames corresponding to the genes *SLS1*, yLR140w, and *RRN5* (Figure 1) and is characterized in this study. In pRMS5-6, none of the ORFs are inserted in a manner that would be predicted to allow overexpression via the *GAL* promoter on the plasmid; therefore suppression is most likely due to increased gene dosage (see materials and methods). To determine which of these genes is responsible for the suppressor activity, we constructed three plasmids that contained subclones of the original genomic insert. The plasmid pRMS5-6Δ1 contains intact *SLS1* and yLR140w ORFs and the plasmid pRMS5-6Δ2 contains only an intact *SLS1* ORF. Each of these plasmids retained suppressor activity (Figure 1). In contrast, a plasmid that harbored only the *RRN5* ORF (pRMS5-6Δ3) did not exhibit sup-

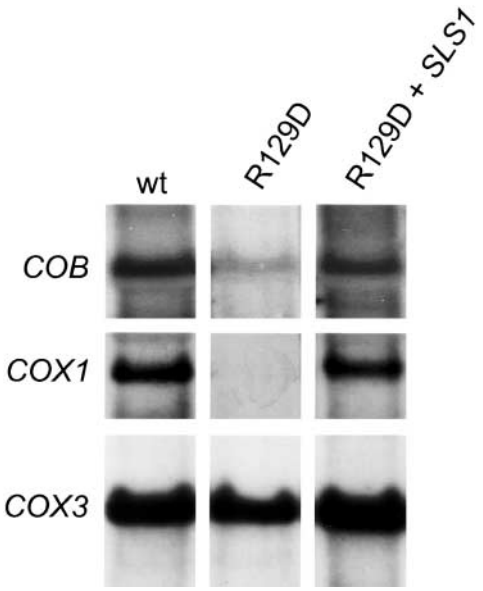

FIGURE 2.—Mitochondrial *COX1* and *COB* transcript defects in a *rpo41-R129D* mutant strain are corrected by overexpression of *SLS1*. Shown is a Northern analysis of total mitochondrial RNA isolated from the following yeast strains after growth at 37° for five generations as described previously (RODEHEFFER *et al.* 2001): GS122, *RPO41* wild type (wt); GS129, *rpo41-R129D* (R129D); GS129 transformed with pRMS5-6 (R129D + *SLS1*). Signals for the mature mitochondrial RNA transcripts *COB*, *COX1*, and *COX3* are indicated on the left.

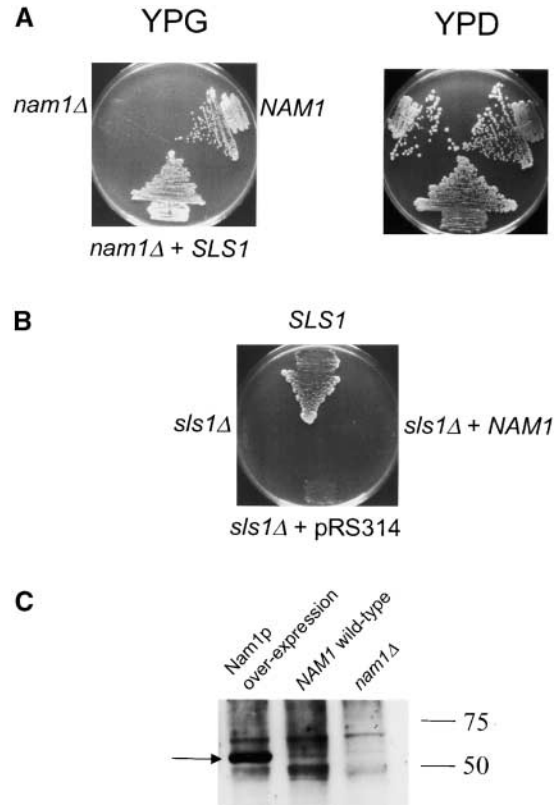

FIGURE 3.—Genetic analysis of *SLS1* and *NAM1* null mutant strains. (A) Overexpression of *SLS1* restores respiration competence to a *NAM1* null strain. Petite phenotypes of the following strains revealed by growth on YPD (respiration nonselective) and YPG (respiration selective) medium at 30°: GS140, *NAM1* wild type (*NAM1*); GS142, *NAM1* null (*nam1*Δ); and ACB1, *NAM1* null + pRS316-*SLS1* (*nam1*Δ + *SLS1*). Labeling of the YPD plate is the same as that indicated for the YPG plate. (B) Overexpression of Nam1p does not restore respiration competence to an *SLS1* null strain. Growth phenotypes of the following strains revealed by growth on YPG-GAL-fluoroorotic acid (respiration selective + galactose) medium at 30°: CMW4, *SLS1* wild type (*SLS1*); CMW2 with no plasmid (*sls1*Δ); CMW3, *SLS1* null + pRS314 (*sls1*Δ + pRS314); and CMW5, *SLS1* null + pRS314-*NAM1*t (*sls1*Δ + *NAM1*). (C) Confirmation that Nam1p is overproduced in CMW5 in the presence of galactose. Shown is a Western blot of total cellular protein (~10 μg) from the following strains probed for Nam1p: left lane, CMW5 (tagged Nam1p overexpression strain) grown in YPG-GAL medium; middle lane, CMW2 + pRS314 (no *NAM1* insert control) grown in YPG-GAL medium; right lane, GS142 (*nam1*Δ control) grown in YPD medium. The position of the tagged-Nam1p signal (~60 kD) is indicated by an arrow and molecular weight standards are indicated on the right.

pressor activity (Figure 1). These data implicated the *SLS1* gene as the determinant of the suppression activity. This assignment is supported by the fact that the *SLS1* gene encodes a mitochondrial membrane protein that is required for normal mitochondrial respiration (ROUILLARD *et al.* 1996).

Rescue of *COX1* and *COB* RNA-processing defects: A documented molecular phenotype of the *rpo41-R129D* mutation, as well as other mutations in the amino-terminal domain of mtRNA polymerase, is a RNA-processing defect that leads to decreased accumulation of mature mitochondrial *COX1* and *COB* transcripts (RODEHEFFER *et al.* 2001). We examined the levels of these two transcripts and a control *COX3* transcript that is not affected by this mutation in the *SLS1*-suppressed strain (RMS5-6) by Northern analysis. The presence of the *SLS1* plasmid (pRMS5-6) restored the steady-state amounts of mature *COX1* and *COB* mRNAs to virtually wild-type levels (Figure 2).

***SLS1* overexpression rescues the petite phenotype of a *NAM1* null mutation:** Because *SLS1* overexpression was able to correct the *COX1* and *COB* transcript defects of the *rpo41-R129D* mutation and these are the same defects observed in *NAM1* null mutant strains (GROUDINSKY *et al.* 1993; RODEHEFFER *et al.* 2001), we determined whether overexpression of *SLS1* can rescue the petite phenotype of a *NAM1* null mutation. We found that overexpression of *SLS1* from pRS316-*SLS1* (a low-copy plasmid that expresses *SLS1* from its own promoter) restored respiration capacity to a *NAM1* null mutant strain GS142 (Figure 3A).

Overexpression of Nam1p does not rescue the petite phenotype of the *SLS1* null mutation: The ability of *SLS1* overexpression to bypass the requirement for Nam1p (Figure 3A) indicated that these two genes are either functionally redundant or acting together in a pathway of events. To begin to distinguish between these two

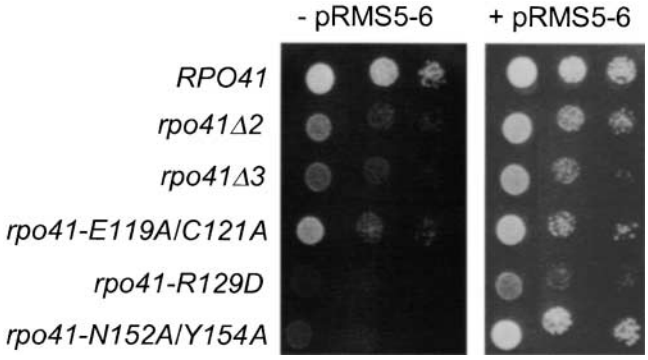

Figure 4.—*SLS1* overexpression suppresses the mitochondrial petite phenotypes of other mtRNA polymerase amino-terminal domain mutations. The following strains with (+pRMS5-6) or without (−pRMS5-6) the *SLS1*-containing suppressor plasmid pRMS5-6 were grown on YPG (respiration selective) or YPD (respiration nonselective) medium at 37°: GS122 (*RPO41*), GS124 (*rpo41*Δ2), GS125 (*rpo41*Δ*3*), GS128 (*rpo41-E119A/C121A*), GS129 (*rpo41-R129D*), and GS130 (*rpo41-N152A/Y154A*). Shown is the growth of serial dilutions (from left to right) of liquid cultures of the indicated strains spotted onto solid medium as described (Rodeheffer *et al.* 2001).

possibilities, we determined whether overexpression of Nam1p can rescue the petite phenotype of a *SLS1* null mutation using a plasmid-shuffle strategy. We found that overexpression of a functional tagged version of Nam1p was incapable of restoring respiration competence to a *SLS1* null strain, CMW5 (Figure 3B). That tagged Nam1p was significantly overproduced under the growth conditions tested was confirmed by Western immunoblot analysis (Figure 3B).

Overexpression of *SLS1* rescues the petite phenotype of other amino-terminal domain mutations: We have identified several mtRNA polymerase amino-terminal domain mutations that exhibit *NAM1*-like mtRNA-processing defects, but display varying abilities to interact with Nam1p in a two-hybrid assay (Rodeheffer *et al.* 2001). We tested the degree to which *SLS1* overexpression suppresses the petite phenotypes of this bank of mutant strains. The presence of the *SLS1*-overexpression plasmid (pRMS5-6) enhanced the YPG growth rates in all of these strains; however, the degree of this suppression was allele specific (Figure 4). Similar to the *rpo41-R129D* mutation, *SLS1* overexpression substantially rescued the severe growth phenotype of the *rpo41-N152A/Y154A* mutation. The remainder of the mtRNA polymerase mutants, which have less severe growth defects, was also rescued, but to a lesser extent relative to the corresponding parental mutant strains (Figure 4). In particular, the *rpo41-E119A/C121A* mutant was only modestly affected by the presence of the *SLS1* plasmid.

DISCUSSION

The amino-terminal domain of yeast mtRNA polymerase is the binding site for Nam1p (Rodeheffer *et al.* 2001) and has been implicated in coupling translation and/or RNA-processing events to transcription in mitochondria (Wang and Shadel 1999). We identified the *SLS1* gene, which encodes a mitochondrial membrane protein (Rouillard *et al.* 1996), as a genetic suppressor of a point mutation in the yeast mtRNA polymerase amino-terminal domain (*rpo41-R129D*) when moderately overexpressed. Several published observations indicate that this genetic interaction between *SLS1* and mtRNA polymerase holds significance for the mechanism of gene expression in yeast mitochondria. First, *NAM1* null mutant strains have phenotypes in common with mtRNA polymerase amino-terminal domain mutants and *SLS1* null mutants. Specifically, *NAM1* null mutations and mtRNA polymerase amino terminal-domain mutations each result in decreased amounts of mature mitochondrial *COX1* and *COB* transcripts (Groudinsky *et al.* 1993; Rodeheffer *et al.* 2001), while *NAM1* null mutations and *SLS1* null mutations each result in reduced amounts of mitochondrial complexes III and IV (Asher *et al.* 1989; Rouillard *et al.* 1996). Altogether, these data implicated Nam1p as a likely intermediate in a pathway of events involving mtRNA polymerase and Sls1p and led us to examine this possibility in greater detail.

We provide here three additional lines of evidence that lead us to conclude that Sls1p is most likely in a pathway of mitochondrial gene expression events with Nam1p and mtRNA polymerase. First, overexpression of *SLS1* rescues the *NAM1*-like *COX1* and *COB* transcript defects manifested in the *rpo41-R129D* strain (Figure 2) and suppresses the petite phenotype of several mutations in the amino-terminal domain of mtRNA polymerase (Figure 4), indicating that Sls1p function impinges directly on the mtRNA polymerase/Nam1p pathway and does not suppress the *rpo41-R129D* phenotype by an unrelated mechanism. Second, overexpression of *SLS1* can fully bypass the function of Nam1p (Figure 3), which is also consistent with a functional link between Nam1p and Sls1p. And third, on the basis of the inability of increased levels of Nam1p to rescue the loss of Sls1p function (Figure 3), we conclude that these two gene products are not functionally redundant, but rather most likely work together in a pathway. The ability of moderate overexpression of *SLS1* (*i.e.*, an extra four to five copies of the gene per cell) to have such dramatic effects on mitochondrial function might suggest that this protein product is normally limited in amounts and regulatory in nature.

Gene expression in mitochondria requires the orderly execution of multiple processes that culminate in the assembly of mtDNA-encoded subunits into the inner mitochondrial membrane. Expression begins with transcription by mtRNA polymerase and is followed by numerous RNA-processing events and translation of mature mRNA species. In yeast, substantial evidence that

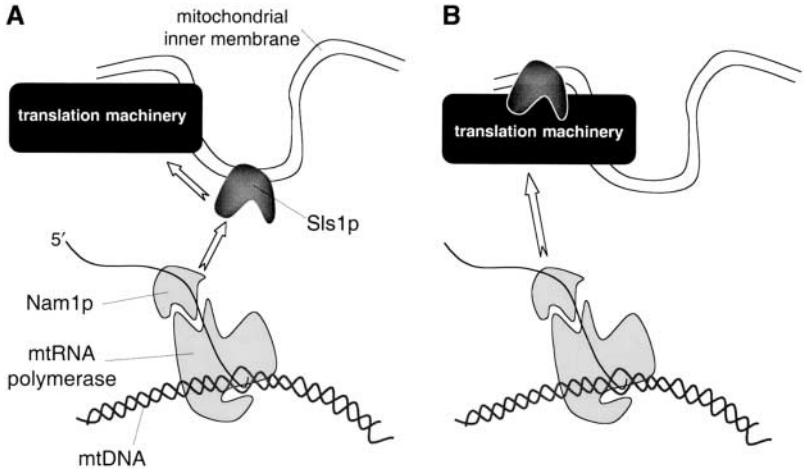

FIGURE 5.—Hypothetical model for the role of Nam1p and Sls1p in mitochondrial gene expression involving an RNA transcript-shuttling pathway from the matrix to the inner membrane. The mitochondrial inner membrane (parallel wavy lines) is shown with components of the mitochondrial translation machinery (*i.e.*, translational activator proteins, ribosomes, etc.) collectively shown as the solid box associated with the membrane. Also shown is Sls1p (shaded) associated with the membrane (ROUILLARD *et al.* 1996) and a mtDNA-bound mtRNA polymerase/Nam1p complex (lightly shaded) with the nascent RNA transcript (single wavy line) drawn emerging from the complex. Two models are proposed, both of which invoke Nam1p as part of a RNA-handling mechanism that, on the basis of its documented interaction with mtRNA polymerase (RODEHEFFER *et al.* 2001), associates with newly synthesized mitochondrial transcripts. The models (A and B) differ with regard to the putative function of Sls1p in the pathway and in the manner in which transcripts are delivered to the translation machinery. (A) Sls1p is part of the membrane-bound segment of an RNA-delivery pathway. In this model, Sls1p accepts RNA transcripts *after* they have been handled by Nam1p and facilitates subsequent delivery of transcripts to the translation machinery. (B) Sls1p is involved in translation efficiency and therefore is part of the translation machinery. In this model, Nam1p is involved in a mechanism to deliver transcripts directly (*i.e.*, not via Sls1p) to the translation machinery and Sls1p functions during subsequent events that facilitate translation of these messages (*e.g.*, loading translational activators or ribosomes onto mRNAs).

indicates translation of mitochondrial mRNAs occurs in association with the inner mitochondrial membrane has accumulated. This includes the localization of gene-specific translational activators (MCMULLIN and FOX 1993; WIESENBERGER and FOX 1997; MANTHEY *et al.* 1998; GREEN-WILLMS *et al.* 2001) and ribosomes (SPITHILL *et al.* 1979) to the membrane and the dependence of translation on membrane lipid composition (MARZUKI *et al.* 1975; OSTRANDER *et al.* 2001). The localization of translation at the membrane possibly coordinates mitochondrial protein synthesis with the insertion of the nascent peptides into the membrane (COSTANZO and FOX 1990) or facilitates assembly of mtDNA-encoded and nucleus-encoded protein subunits into the higher-order complexes of the respiratory chain (SANCHIRICO *et al.* 1998). Our results strongly suggest that gene expression in mitochondria is also regulated at the RNA level prior to and/or during these membrane-associated events. We propose a model (Figure 5) that invokes a pathway of RNA-handling events that orchestrates the delivery of newly synthesized RNA transcripts to the membrane, where they are subsequently outfitted for translation. In this model, Nam1p, through its interaction with mtRNA polymerase (RODEHEFFER *et al.* 2001), is predicted to interact directly or to facilitate interactions with nascent RNA transcripts and to promote their transit to the membrane, a function for Nam1p similar to that speculated by others (WALLIS *et al.* 1994; MANTHEY *et al.* 1998). Because it is a mitochondrial membrane protein (ROUILLARD *et al.* 1996), we postulate that Sls1p either is part of a membrane-associated RNA-shuttling mechanism that helps deliver Nam1p- associated transcripts to the translation machinery (Figure 5A) or is itself intimately involved with the translation machinery (Figure 5B). In this latter scenario, Nam1p-associated transcripts are delivered to the translation machinery directly and Sls1p promotes their translation in some other manner (*e.g.*, by facilitating the loading or activity of translational activator proteins or ribosomes at the membrane). An underlying prediction of this model is that a primary function of both Sls1p and Nam1p is translational regulation and that some aspects of translation are coupled to transcription in mitochondria. In the case of Nam1p, a function in mitochondrial translation has already been documented (ASHER *et al.* 1989). In addition, mitochondrial intron-splicing events, which are dependent upon translation, are very sensitive to reductions in translation efficiency (ZHANG *et al.* 2000). This suggests that the intron-containing *COX1* and *COB* transcript defects observed in *NAM1* null and mtRNA polymerase amino-terminal domain mutant strains are likely a secondary effect of decreased translational efficiency. Therefore, it follows that the ability of Sls1p to correct these same transcript defects in the *rpo41-R129D* strain (Figure 2) could be explained by its ability to modulate translation efficiency by some mechanism. Finally, another documented readout of decreased translation efficiency in yeast mitochondria is mtDNA instability (SHADEL 1999). Again, consistent with an involvement in translation is the observation that mutations in *SLS1*, *NAM1*, and the mtRNA polymerase amino-terminal domain all result in mitochondrial genome instability (ASHER *et al.* 1989; ROUILLARD *et al.* 1996; WANG and SHADEL 1999). Altogether, these data support our proposed model (Figure 5) that predicts that the downstream effects of mutations in

the amino-terminal domain of mtRNA polymerase (and *NAM1* null mutations; Asher *et al.* 1989) ultimately cause reductions in mitochondrial translation and that overexpression of *SLS1*, by facilitating the utilization of transcripts by the translation machinery, can rescue these defects. Important avenues of future investigation include deciphering the precise role of Nam1p and Sls1p in shuttling RNA or regulating translation and the degree to which translation and transcription are functionally coupled during mitochondrial gene expression.

Our results provide new insight into the general mechanism of mitochondrial gene expression and indicate that multiple levels of regulation exist. While, on the surface, it appears that the mechanism of translation in yeast mitochondria is markedly different from that in mammals (*e.g.*, RNAs lack long 5′-UTRs and homologs of Nam1p, Sls1p, and the translational activators apparently do not exist), it is noteworthy that mitochondrial ribosomes have recently been reported to be membrane-associated in bovine cells (Liu and Spremulli 2000). This implies the need for a mechanism to deliver RNA transcripts to the membrane in mammalian mitochondria as well. That such a mechanism might bear some resemblance to the putative yeast mitochondrial RNA-handling pathway we propose in this report is suggested by the fact that the human and Xenopus mtRNA polymerase amino-terminal extensions contain a PPR motif (Rodeheffer *et al.* 2001), a conserved domain found in numerous proteins involved in RNA interactions (Small and Peeters 2000). Thus, as in yeast, an amino-terminal domain of human mtRNA polymerase may be the nucleation point for important RNA-handling pathways involved in mitochondrial gene expression. Characterizing these pathways will likely be important for understanding mitochondrial gene expression in humans and the pathology of mitochodrial-related diseases.

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