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

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Manuscript received August 16, 2001
Accepted for publication October 18, 2001

**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 mRNA 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 respiratory 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; Smittik et al. 2001). Currently, our knowledge of mitochondrial genomics 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-
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-Wilms 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 XbaI-BamHI 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 Smal-BclI fragment and ligated into the Smal and SpeI sites in pRS314 to create the plasmid pRS314-NAM1V5. 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 RMS6-6 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: pRMS5-6a, a ~3-kb XbaI fragment containing intact SLS1 and yLR140w ORFs, was ligated into pRS316; pRMS5-6b, a ~2.2-kb XbaI-SpeI fragment containing only an intact SLS1 ORF, was ligated into pRS316; and pRMS5-6c, a ~2-kb SpeI-DraI 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 (wild-type, background; see Figure 1). Construction of yeast strain GS122 has been described (Wang and Shaedel 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 HIN3 cassette that was inserted at the Snal and XbaI 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-NAM1, respectively.

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 plasm-
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 YES-R library and Urα transforms were selected as described previously (et al. 2001), except the blot was probed this study. In pRMS5-6, none of the ORFs are inserted with a rabbit polyclonal antibody...in a manner that would be predicted to allow overexpression of the gene (in a respiration-competent, but slow-growth, phenotype...promoter on the plasmid; therefore suppression is most likely due to increased gene expression via the... Because the mutant strain (GS129) was identified. Three of these plasmids contained the phenotype was plasmid linked. This plasmid (pRMS5-6) was isolated and the genomic DNA fragment in each plasmid is main-...one strong suppressor strain R129D was identified. Three of these plasmids contained the 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 mRNA and detection of mature COX1, COB, and COX3 messages was performed as described previously (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 mRNA polymerase amino-terminal domain mutation rpo41-R129D: In a previous study (et al. 2001), we characterized mutations in the amino-terminal domain of yeast mRNA 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 MATα 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 RNR5 (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 RNR5 ORF (pRMS5-6Δ3) did not exhibit sup-

### TABLE 1

<table>
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<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
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<tr>
<td>GS128</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 rpo41Δ1::HIS3 + [pRS314-rpo41-E119A/ C121A]</td>
<td>Rodeheffer et al. (2001)</td>
</tr>
<tr>
<td>GS129</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 rpo41Δ1::HIS3 + [pRS314-rpo41-R129D]</td>
<td>Rodeheffer et al. (2001)</td>
</tr>
<tr>
<td>GS130</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 rpo41Δ1::HIS3 + [pRS314-rpo41-N152A/ Y154A]</td>
<td>Rodeheffer et al. (2001)</td>
</tr>
<tr>
<td>GS140</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 nam1Δ::HIS3 + [pRS316-NAM1]</td>
<td>This study</td>
</tr>
<tr>
<td>GS141</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 nam1Δ::HIS3 + [pRS314-NAM1]</td>
<td>This study</td>
</tr>
<tr>
<td>GS142</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 nam1Δ::HIS3 + [pRS314]</td>
<td>This study</td>
</tr>
<tr>
<td>ACB1</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 nam1Δ::HIS3 + [pRS314] + [pRS316-SLS1]</td>
<td>This study</td>
</tr>
<tr>
<td>CMW2</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 sls1Δ::KanMX4 + [pRS316-SLS1]</td>
<td>This study</td>
</tr>
<tr>
<td>CMW3</td>
<td>ahis3Δ200 leu2-3,112 ura3-52 trp-1Δ1 ade2 sls1Δ::KanMX4 + [pRS314]</td>
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</tr>
<tr>
<td>CMW4</td>
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<td>This study</td>
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<tr>
<td>CMW5</td>
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<td>This study</td>
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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°C 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°C: GS140, NAM1 wild type (NAM1); GS142, NAM1 null (nam1Δ); and ACB1, NAM1 null + pRS316-SLS1 (nam1Δ + 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°C: CMW4, SLS1 wild type (SLS1); CMW2 with no plasmid (sls1Δ); CMW3, SLS1 null + pRS314 (sls1Δ + pRS314); and CMW5, SLS1 null + pRS314-NAM1 (sls1Δ + 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 (nam1Δ 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
Mitochondrial Gene Expression Pathway

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 mtDNA polymerase amino-terminal domain mutations that exhibit NAM1-like mtDNA-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 mtDNA 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 mtDNA 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 mtDNA polymerase amino-terminal domain (rpo41-R129D) when moderately overexpressed. Several published observations indicate that this genetic interaction between SLS1 and mtDNA polymerase holds significance for the mechanism of gene expression in yeast mitochondria. First, NAM1 null mutant strains have phenotypes in common with mtDNA polymerase amino-terminal domain mutants and SLS1 null mutants. Specifically, NAM1 null mutations and mtDNA 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 mtDNA 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 mtDNA 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 mtDNA polymerase (Figure 4), indicating that Sls1p function impinges directly on the mtDNA 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 mtDNA polymerase and is followed by numerous RNA-processing events and translation of mature mRNA species. In yeast, substantial evidence that
transcripts. The models (A and B) differ with regard to the putative function of Sls1p in the pathway 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 genespecific 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 mitochondrial 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 intronsplicing 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 NAMI 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 pathologies of mitochondrial-related diseases.

The authors thank Dr. David A. Clayton for providing the Nam1p antibody used in this study. This work was supported by grant HL-59655 from the National Institutes of Health awarded to G.S.S.

LITERATURE CITED


SPREMULLI, T. W., P. NAGLEY and A. W. LINNANE, 1979 Biogenesis of mitochondria 51: biochemical characterization of a mitochon-


Wallis, M. G., O. Groudinsky, P. P. Slonimski and G. Dujardin, 1994 The NAM1 protein (NAM1p), which is selectively required for cox1, cytb and atp6 transcript processing/stabilisation, is located in the yeast mitochondrial matrix. Eur. J. Biochem. 222: 27–32.

