Nam1p, a Protein Involved in RNA Processing and Translation, Is Coupled to Transcription through an Interaction with Yeast Mitochondrial RNA Polymerase*

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Alignment of three fungal mtRNA polymerases revealed conserved amino acid sequences in an amino-terminal region of the \textit{Saccharomyces cerevisiae} enzyme implicated previously as harboring an important functional domain. Phenotypic analysis of deletion and point mutations, in conjunction with a yeast two-hybrid assay, revealed that Nam1p, a protein involved in RNA processing and translation in mitochondria, binds specifically to this domain. The significance of this interaction in vivo was demonstrated by the fact that the temperature-sensitive phenotype of a deletion mutation (\textit{rpo41Δ2}), which impinges on this amino-terminal domain, is suppressed by overproducing Nam1p. In addition, mutations in the amino-terminal domain result specifically in decreased steady-state levels of mature mitochondrial \textit{CYTB} and \textit{COXI} transcripts, which is a primary defect observed in \textit{NAM1} null mutant yeast strains. Finally, one point mutation (\textit{R129D}) did not abolish Nam1p binding, yet displayed an obvious \textit{COXI}/\textit{CYTB} transcript defect. This mutation exhibited the most severe mitochondrial phenotype, suggesting that mutations in the amino-terminal domain can perturb other critical interactions, in addition to Nam1p binding, that contribute to the observed phenotypes. These results implicate the amino-terminal domain of mtRNA polymerases in coupling additional factors and activities involved in mitochondrial gene expression directly to the transcription machinery.

Expression of the mitochondrial genome occurs in the organelle matrix and involves both nuclear- and mtDNA-encoded factors. In addition to mRNAs (which usually encode protein subunits of the enzyme complexes involved in oxidative phosphorylation), mtDNA in many organisms also contains genes for tRNAs and ribosomal RNAs that are necessary for mitochondrial translation. The remaining factors required for expression and replication of the mitochondrial genome are encoded in the nucleus and imported into the organelle. Mitochondrial transcripts are often polycistrionic and thus a large number of RNA processing reactions is required to liberate mature RNA species (1). In addition, RNA processing events have been implicated in the initiation of mtDNA replication (2). Thus, a complete understanding of mitochondrial gene expression and mtDNA replication requires broader understanding of how these RNA processing events are accomplished in vivo.

The mitochondrial transcription machinery in the budding yeast, \textit{Saccharomyces cerevisiae}, is well characterized (3, 4) and involves a nucleus-encoded mtRNA polymerase (sc-mtRNA polymerase, encoded by the \textit{RPO41} gene) that is homologous to the single subunit \textit{Escherichia coli} bacteriophage RNA polymerase (5, 6) and a transcription initiation factor sc-mtTFB (7–9). In addition, factors involved in mtRNA processing have also been identified in this organism, including those involved in liberating tRNAs, rRNAs, and mRNAs from polycistrionic transcripts and excising introns from certain messages (see Ref. 10 for review). One such factor, Nam1p (also known as Mlt2p), was initially identified as a high copy suppressor of mtDNA point mutations that affect splicing of introns from the mitochondrial \textit{COXI} and \textit{CYTB} messages (11) and independently as a temperature-sensitive mutation affecting mitochondrial transcripts (12). Nam1p is localized to the mitochondrial matrix (13), and characterization of \textit{NAM1} null mutant strains has confirmed its involvement in \textit{COXI} and \textit{CYTB} intron removal and elucidated potential roles for this protein in overall mitochondrial translation capacity and \textit{ATP6}/\textit{8} mRNA processing and/or stability (14). In addition, crude mitochondrial transcription complexes isolated from one \textit{NAM1} mutant strain remain competent for transcription but exhibit an altered RNA binding activity profile (12), suggesting a potential link between Nam1p function and the mitochondrial transcription machinery.

We had shown previously that an amino-terminal domain of yeast mtRNA polymerase is dispensable for transcription initiation in vivo but nonetheless is required for stability and maintenance of the mitochondrial genome, suggesting that additional activities may be coupled to the transcription process in mitochondria (15). These studies implicated amino acids 29–208 of mtRNA polymerase as a minimal portion of the protein that harbors an independent functional domain of the enzyme. Here we have characterized this domain further and demonstrate that one of its functions is to provide an interaction point for Nam1p, that may provide the means to couple factors involved in additional aspects of RNA metabolism directly to the transcription machinery in yeast mitochondria. In principal, such a coupling phenomenon in mitochondria may be analogous to mechanisms of gene expression in the nucleus, where many aspects of mRNA processing are coupled functionally to transcription via interactions involving the carboxyl-terminal domain of the largest subunit of RNA polymerase II (16, 17).

* This work was supported by NHLBI Grant HL-59655 from the National Institutes of Health (to G. S. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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EXPERIMENTAL PROCEDURES

Plasmid Construction—Wild-type and mutated alleles of RPO41 used in the plasmid shuffle assay were expressed in yeast from the plasmid pGS348 (15), which contains a 7.2-kb, RPO41 gene-containing, SalI-SpeI restriction fragment of yeast genomic DNA cloned into the shuttle vector pRS314 (18). Construction of the rpo41Δ2 and rpo41Δ3-containing versions of pGS348 has been described elsewhere (15). Two-hybrid bait plasmids were utilized in this study. Derivatives of pAS1 (19), which express the RPO41 reading frame as amino-terminal fusions to the Gal4p DNA-binding domain. Two parental RPO41-bait plasmids were used, pAS1-RPO and pAS1-RPO-FL, that contained amino acids 27–633 and amino acids 27–1351 of sc-mtRNA polymerase, respectively (numbered according to Ref. 6). To construct the corresponding mutated RPO41-bait plasmids, restriction fragments containing the mutated versions of RPO41 were isolated from PCR products (or from the corresponding pGS348 derivatives) and ligated into either pAS1-RPO or pAS1-RPO-FL (Table I). The plasmid used to overproduce Nam1p (pYES/GS-NAM1) was obtained from Invitrogen (Genetools clone yD0L44Cv). This plasmid contains a V5 epitope-tagged version of the NAM1 gene under control of a galactose-inducible promoter.

Site-directed Mutagenesis—Specific point mutations in the RPO41 gene were generated by a two-step, megaprimer PCR protocol as follows. The PCR template (pGS348) consisted of a 2.1-kb SalI-BamHI restriction fragment from pGS348, which contains the amino-terminal extension of RPO41 and upstream sequences, cloned into pBluescript KS+ (Stratagene) vectors for transforming yeast DNA polymerase gene as their only source of mtRNA polymerase, respectively (numbered according to Ref. 6). To characterize the growth phenotypes of rottoronic acid were used to select for strains that express the mutated chain reaction; aa, amino acids.

2.2-kb product of this second PCR, which now had the desired mutation during the PCR procedure. The PCR template (pBS348) consisted of a 2.1-kb SalI-BamHI restriction fragment in this plasmid. For each mutation, a PCR was performed that utilized a specific mutagenic oligonucleotide (synthesized by Mid-land Certified Reagent Co., Midland, TX) as one primer and an oligonucleotide corresponding to the T7 promoter as the second primer to generate an ~500-base pair product. This PCR product was gel-purified and used as the source of a megaprimer (after denaturation, the 500-base pair product was gel-purified and used as the megaprimer) in a second PCR in conjunction with a T3 primer. The 2.2-kb product of this second PCR, which now had the desired mutation fixed in the RPO41 gene, was digested with SalI and BamHI and ligated into pGS348 to form a full-length mutated RPO41 allele that can be expressed in yeast. All PCRs were performed using Pfu Turbo DNA Polymerase (Stratagene, Inc.) in the buffer supplied by the manufacturer and typically consisted of 25–30 amplification cycles (95 °C, 30 s; 55 °C, 1 min; 68 °C, 8 min) followed by a 12-min, 68 °C extension period at the end of the last cycle. In all cases, the nucleotide sequence of the portion of RPO41 open reading frame in pGS348 that was amplified during the mutagenesis protocol was determined to ensure that the desired site-directed mutation was the only mutation introduced during the PCR procedure.

Phenotypic Analysis of RPO41 Mutations by Plasmid Shuffle—Routine growth and transformation of yeast strains, as well as standard growth media preparation, were accomplished as described by Sherman (20). The yeast strain GS112 (α his3-d200 leu2-3,-112 ura3-52 trp1-901 ade2-101 ura3-52 leu2-3,-112 + URA3; GAL-LacZ + LYS2::GAL-HIS3 cya-) was used to analyze RPO41 gene mutations by plasmid shuffle (21). After transformation of GS112 with the desired mutant RPO41 allele on a pGS348 plasmid, two rounds of growth on synthetic dextrose (SD) medium containing 5-fluoroorotic acid were used to select for strains that expressed the mutated version of the RPO41 gene as their only source of mtRNA polymerase, as described previously (15). To characterize the growth phenotypes of the mutant strains in detail, serial 10-fold dilutions were plated onto solid YPD (glucose-containing medium) and YPG (glycerol-containing medium) using a 48-pin multiplex plating tool (“Frogger,” Dankar, Inc.) and grown at both 30 and 37 °C. Growth on YPG medium requires mitochondrial respiration, whereas growth on YPD does not; therefore, defective growth on YPG medium was scored as a mitochondrial petite phenotype.

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RESULTS

A Conserved Amino-terminal Domain in sc-mtRNA Polymerase—The mtRNA polymerase of S. cerevisiae is related to bacteriophage RNA polymerases (e.g. T7, T3, and SP6) but contains a unique amino-terminal extension (Fig. 1). In an earlier study (15), we reported the analysis of a series of RPO41 deletion mutations that had no effect on protein stability or localization, and ultimately revealed the existence of a functional amino-terminal domain in mtRNA polymerase between amino acids 28 and 208 that is required for mtDNA maintenance and can function in trans. Whereas mtRNA polymerases from most species contain significant amino acid identity in those regions involved in catalytic activity (i.e. the bacteriophage T7 family-like domains), the amino-terminal extensions 1
are not well conserved between species. However, we were able to align the amino acid sequence of the amino-terminal extension of the S. cerevisiae enzyme with that from two other fungal species, Neurospora crassa and Schizosaccharomyces pombe, and found that the most conserved region corresponded to amino acids 110–205 of the S. cerevisiae mtRNA polymerase (Fig. 1). In particular, the region encompassing amino acids 117–155 exhibited 18% amino acid identity (identical residues in all three species) and 38% amino acid similarity (identical or similar amino acids in all three species). The degree of similarity in this region is emphasized further if pairwise comparisons are made. For example, the S. cerevisiae and N. crassa proteins are 35% identical and 56% similar in this region.

To test the hypothesis that the conserved region of the mtRNA polymerase amino-terminal extension composes all or part of the important functional domain (aa 28–208) we identified previously (15), we made a series of point mutations in the wild-type protein. Again, these results are consistent with our earlier analysis of the rpo41Δ3 mutation that removes this domain completely but does not affect protein localization or stability (15).

Nam1p Interacts with the Amino-terminal Domain of Yeast mtRNA Polymerase—The observed phenotypes of the amino-terminal domain mutations suggest that this region of the protein is involved in coupling some critical function to the transcription process in mitochondria (15). This coupling capacity could occur directly, by virtue of a structural or catalytic role for this domain, or indirectly through the binding of other mitochondrial regulatory factors, or perhaps both. To test the hypothesis that other mitochondrial factors are involved, we screened a library of yeast genomic DNA fragments for proteins that bind to the amino-terminal portion of yeast mtRNA polymerase that was implicated here and in our previous studies (15). To accomplish this, we created

**Fig. 1.** A region of the sc-mtRNA polymerase amino-terminal extension is conserved in two other fungal species. A linear representation of sc-mtRNA polymerase (encoded by the RPO41 gene) is presented at the top of the figure. The carboxyl-terminal portion of the enzyme that is homologous to bacteriophage RNA polymerases is depicted as a gray box, the mitochondrial targeting sequence by a black box, and the amino-terminal extension by a white box. Expanded at the bottom is an alignment of the most conserved portion of the amino-terminal extensions from S. cerevisiae (S.c.), amino acids 110–205; N. crassa (N.c.), amino acids 122–219; and S. pombe (S.p.), amino acids 34–117. Amino acid residues that are identical are indicated by darker shading, and those that are similar are indicated by lighter shading. The residues in sc-mtRNA polymerase that were changed by site-directed mutagenesis are indicated by arrows, at the ends of the arrows the amino acid substitution is shown (as well as the RPO41 allele designations, which are boxed). Also indicated is the end point of two deletion mutations that were characterized previously (15). The rpo41Δ2 allele deletes amino acids 27–117 (end point labeled Δ2), and thus partially impinges on the conserved region. The rpo41Δ3 allele deleted amino acids 27–212 (end point labeled Δ3) and therefore completely removes the conserved region.
two yeast two-hybrid bait plasmids. The first, pAS-RPO, contained \textit{RPO41} sequences that encompassed an intact amino-terminal domain (encoding aa 27–633) and the second, pAS-RPOΔ4, that does not encode the amino-terminal domain but does contain other \textit{RPO41} sequences (encoding aa 392–633). The screen involved several steps that ultimately selected for plasmids encoding proteins that exhibited an interaction specifically with the amino-terminal portion of sc-mtRNA polymerase (see “Experimental Procedures”). From an initial screen focused our attention on the amino-terminal domain altogether, did not interact in this region. A, \(\beta\)-galactosidase activity (dark color in lower panel) indicates a two-hybrid interaction between Nam1p and sc-mtRNA polymerase in the lacZ-reporter strain Y190, which contains a plasmid (pAS1-RPO) encoding an intact amino-terminal domain. The same strain containing a plasmid (pAS-RPOΔ4) that is missing the amino-terminal domain, but contains other sc-mtRNA polymerase sequences, produces no \(\beta\)-galactosidase activity (upper panel, no dark color). B, overproduction of Nam1p suppresses the YPG growth defects of a rpo41Δ2 strain but not a rpo41Δ3 strain. Five strains that were streaked onto a YPG plate and grown at 36 °C are shown. The yeast strain GS124 contains a plasmid encoding the rpo41Δ2 allele as its only source of sc-mtRNA polymerase; the yeast strain GS125 contains a plasmid encoding the rpo41Δ3 allele as its only source of sc-mtRNA polymerase; and GS122 is the isogenic wild-type strain that has the wild-type \textit{RPO41} gene provided on a plasmid as its only source of sc-mtRNA polymerase (15). GS124 and GS125 transformed with an empty \textit{URA3} plasmid (YEp352) or with a \textit{URA3} plasmid that overexpresses Nam1p (pYES/GS-NAM1) are indicated.
Nam1p to some degree in this assay. All of the mutated RPO41 fusion proteins shown in Table I were expressed at least as well as the wild-type fusion protein (data not shown); thus those mutations reported to disrupt the Nam1p interaction did not dramatically affect expression or stability of the two-hybrid bait protein.

Nam1p Overexpression Rescues the Temperature-sensitive Phenotype of the rpo41Δ2 Mutation—To determine whether the Nam1p interaction with the amino-terminal domain of mtRNA polymerase that we identified by two-hybrid analysis is of physiological significance, we next tested whether overexpression of Nam1p could rescue the phenotype of mutations in the amino-terminal domain. To accomplish this we utilized a high copy plasmid (pYES/GS-NAM1) that expresses an epitope-tagged version of the NAM1 gene from a galactose-inducible promoter (see “Experimental Procedures”). Expression levels of the tagged version of Nam1p from this promoter, even without galactose induction, are capable of complementing a chromosomal NAM1 disruption, confirming that the tagged Nam1p is functional in vivo (data not shown). Introduction of this Nam1p-overproducing plasmid resulted in significant rescue of the temperature-sensitive phenotype of the rpo41Δ2 mutation but not that of the rpo41Δ3 mutation (Fig. 3B). These results are consistent with the fact that the rpo41Δ2-encoded mtRNA polymerase is still capable of interacting with Nam1p to some extent in the two-hybrid assay, whereas that encoded by rpo41Δ3 is not (Table I). None of the amino-terminal domain point mutations was suppressed by overproducing Nam1p under these conditions (data not shown). In the case of the N152A/Y154A protein, this result is consistent with an inability to interact with Nam1p in the two-hybrid assay. However, the E119A/C121A and R129D proteins still interact with Nam1p to some degree in the two-hybrid assay, yet their mitochondrial defects cannot be rescued by overexpression of Nam1p, suggesting that there are other defects that are contributing to the observed phenotypes of these mutations (see “Discussion”).

Mutations in the Amino-terminal Domain of mtRNA Polymerase Result in Mitochondrial RNA Transcript Defects Consistent with Perturbation of Nam1p Function—A documented phenotype of NAM1 null mutations (14) is the specific reduction of mitochondrial transcripts at that time. To address whether mutations in mtRNA polymerase amino-terminal domain exhibit a nam1-like phenotype, we analyzed mitochondrial transcripts in these strains by northern analysis (Fig. 4). Total mtRNA was analyzed from each strain after growth at 37 °C for five generations and compared with a NAM1 null strain grown at 30 °C, which exhibits the diagnostic COX1 and CYTB transcript defects. As observed in the NAM1 null strain, all of the mtRNA polymerase mutant strains exhibited a marked reduction in the steady-state levels of both CYTB and COX1 messages. In contrast, the steady-state level of COX3, a mitochondrial transcript that is largely unaffected by Nam1p function (14), was not dramatically altered under these conditions and served as an indicator that mitochondrial transcription per se was not globally affected by these mutations. This conclusion is supported by the additional observation that the overall mitochondrial transcript profile in all of the strains, as judged by ethidium bromide staining (data not shown), was virtually identical.

**DISCUSSION**

Amino-terminal extensions present in many mtRNA polymerases distinguish them from the related bacteriophage enzymes and provide the mitochondrial enzymes with localization information as well as additional function (6, 15). Previously, we demonstrated that the amino-terminal extension of sc-mtRNA polymerase harbors a functional domain involved in mtDNA stability, and we proposed that this domain could function by coupling additional activities to the transcription process (15). Because numerous RNA processing events are required for normal mitochondrial gene expression and mtDNA replication (1, 2), we hypothesized (15) that one process that may be coupled to transcription may be RNA processing. Here we report the characterization of a collection of RPO41 deletion and point mutations, the results of which demonstrate that Nam1p, a mitochondrial matrix protein implicated in translation and RNA processing events (11, 13, 14), interacts physically and functionally with an amino-terminal domain of sc-mtRNA polymerase. By using a two-hybrid protein interaction assay, we have demonstrated that Nam1p binds specifically to the amino-terminal extension of sc-mtRNA polymerase. The fact that the rpo41Δ2 mutation did not abolish Nam1p binding in this assay, whereas the rpo41Δ3 mutation did, suggests that the primary binding site being assayed here lies between the end points of these two deletions (amino acids 118–208). Consistent with this proposal, alignment of the amino-terminal extensions of *S. cerevisiae*, *N. crassa*, and *S. pombe* revealed that, although not highly conserved overall, the region exhibiting the highest degree of identity/similarity corresponds to amino acids 117–155 of the *S. cerevisiae* protein (Fig. 1).
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The amino-terminal extensions of vertebrate mtRNA polymerases have a repetitive amino acid motif similar to that found in CRP1, a protein involved in RNA-processing and translation in chloroplasts. The human mtRNA polymerase is diagrammed at the top in the same manner as the yeast enzyme in Fig. 1. A linear representation of the Zea mays CRP1 protein is also presented. The region of amino acid sequence similarity that is common to vertebrate (human and Xenopus) mtRNA polymerases and CRP1 is depicted as a hatched box. Shown at the bottom is a ClustalW (30) alignment of two regions in CRP1 (CRP-box1 and CRP-box2) with the analogous region of the human and Xenopus mtRNA polymerases. Black-boxed letters denote amino acid identity, and gray-boxed letters indicate amino acid similarity. Recent evidence suggests that these regions of CRP1 are composed of a tandemly repeated, 35-amino acid domain called a PPR motif that appears to define a new family of proteins involved in RNA processing and translation in organelles (29). Based on a proposed consensus sequence for a PPR motif (29), it appears that the vertebrate mtRNA polymerases contain at least two PPR repeats in the amino-terminal extension (indicated at the bottom of the figure).

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This study also provides additional information regarding the function of the amino-terminal domain of mtRNA polymerase. First, none of the point mutations was rescued by overexpression of Nam1p, despite the ability of some of these to interact with Nam1p, but exhibited the most severe mitochondrial phenotype (capable only of slow growth on YPG at 30 °C, Fig. 1A). All of these data suggest that mutations that affect the amino-terminal domain of mtRNA polymerase lead to mtRNA transcript defects that are entirely consistent with disruption of Nam1p function. The simplest explanation for these data is that at least one function of Nam1p is carried out in association with mtRNA polymerase (i.e. Nam1p is coupled to transcription). The fact that COX3 transcripts are not grossly affected in our experiments is consistent with previously published data showing that mitochondrial transcription per se is not perturbed to large degree by loss of Nam1p function (11, 14) or by the rpo41Δ3 mutation (15).

This study also provides additional information regarding the function of the amino-terminal domain of mtRNA polymerase. First, none of the point mutations was rescued by overexpression of Nam1p, despite the ability of some of these to interact with Nam1p to some degree in the two-hybrid assay (Table I). Second, the R129D and N152A/Y154A mutations exhibited more severe YPG growth phenotypes than the rpo41Δ3 mutation (Fig. 2A), which is completely devoid of this region (Fig. 1). In fact, the R129D mutation was still capable of binding the primary RNA transcript during transcription-coupled processes in mitochondria. In particular, Nam1p appears to be involved in the processing and/or stability of the two intron-containing primary transcripts that contain COX1 and CYTB and, perhaps to a lesser degree, the 21 S tRNA (11, 14). However, Nam1p function is not limited to effects on intron removal because strains that contain an intronless mitochondrial genome still display a temperature-sensitive petite phenotype and instability of the mature ATP6 mRNA (14), which is co-transcribed with COX1. Finally, overall translation capacity is reduced in NAM1 null mutant strains (11), suggesting a dual role for Nam1p in RNA processing/stability and translation, or that these two processes are coupled in yeast mitochondria. Regardless of the precise activity of Nam1p in mtRNA metabolism, our data demonstrate that mutations that affect the amino-terminal domain of mtRNA polymerase lead to mtRNA transcript defects that are entirely consistent with disruption of Nam1p function. The simplest explanation for these data is that at least one function of Nam1p is carried out in association with mtRNA polymerase (i.e. Nam1p is coupled to transcription). The fact that COX3 transcripts are not grossly affected in our experiments is consistent with previously published data showing that mitochondrial transcription per se is not perturbed to large degree by loss of Nam1p function (11, 14) or by the rpo41Δ3 mutation (15).

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scription, perhaps to facilitate threading of the RNA into a coupled RNA-processing machinery during transcription. In this regard, it is noteworthy that the amino-terminal domain of T7 RNA polymerase, the closest relative to mtRNA polymerases, has been implicated in nascent RNA binding (25). If this is the case, then mutations in the amino-terminal domain could affect Nam1p binding, RNA binding, or both, resulting in the more severe phenotypes observed with particular point mutations. An alternative explanation is that a higher order complex is involved that contains not only mtRNA polymerase and Nam1p but also other factors involved in RNA processing/stability or translation. If this were the case, one could envision how point mutations result in the observed phenotypes by disrupting the binding of Nam1p, the binding of other factors, or both. Perhaps consistent with this idea is the observation that splicing of the group I intron (b15) in the mitochondrial CYTB gene is facilitated by the Cbp2 protein in a transcription-dependent manner in vitro (26), suggesting that other factors involved in mtRNA processing events may indeed function in a transcription-coupled manner in vivo. Determination of the precise composition of the Nam1p-mtRNA polymerase complex and assignment of additional functions to the amino-terminal domain remain important goals.

Based on the apparent homology between the amino-ter-
minal domain of sc-mtRNA polymerase and those of N. crassa and S. pombe (Fig. 1), we would predict that these organisms also contain a Nam1p homolog that is localized to the mitochondrial matrix. However, our searches of currently available data bases have yet to identify any obvious homologs of Nam1p in any organism. This brings into question whether the amino-
terminal extensions found in mtRNA polymerases from other eukaryotes have similar functions to that proposed here for the S. cerevisiae protein. To begin to address this question, we have analyzed the sequences of the amino-terminal extensions of human (27) and Xenopus (GenBank™ accession number AF200705) mtRNA polymerases. As already mentioned, no obvious similarity exists between the vertebrate and the fungal enzymes in their amino-terminal extensions. However, like the fungal enzymes, the vertebrate proteins are similar to each other in this region (Fig. 5), exhibiting 34% amino acid identity and 53% similarity. Comparing these conserved amino-ter-
minal regions of the vertebrate homologs to other known se-
quences in available data bases, we found a match of potential significance to a protein, CRP1, that is located in maize chloroplasts (28). Remarkably, the function of CRP1 in chloroplasts is similar to that documented for Nam1p in yeast mitochondria, that is, the processing and translation of specific mRNAs. The conserved amino-terminal region in both the human and Xeno-
opus mtRNA polymerase is similar (20–25% identity, 40–45% similarity) to a block of amino acids that is repeated twice in CRP1 (Fig. 5). Similar data base searches reported by Fisk et al. (28), revealed similarity between CRP1 and a family of plant proteins of unknown function that are related to a salt-induc-
ible protein in tobacco. Additionally, they found that the region held in common between CRP1 and this family of proteins was also related, although more distantly, to several other factors involved in post-transcriptional gene regulation in mitochondria including Pet309p, a translational activator; Rpm2p, a protein subunit of mitochondrial RNaseP (a tRNA processing factor); and threonyl-tRNA synthetase. Recently, the homolo-
gous regions in these proteins have been postulated to be composed of a tandemly repeated, 35-amino acid domain called a pentatricopeptide repeat (PPR) motif that is structurally similar to the well characterized tetratricopeptide repeat (TPR) motif (29). Based on comparisons with a proposed consensus sequence for a PPR motif (29), it appears that vertebrate mtRNA polymerases contain at least two PPR repeats in the amino-terminal extension (Fig. 5). Thus, the human and Xeno-
pus mtRNA polymerases join this list of organelle regulatory proteins involved in RNA processing and translation that likely define a new PPR family of proteins. Although the function of these CRP1-like sequences in vertebrate mtRNA polymerases is unknown, it is interesting to speculate based on our results regarding the function of the S. cerevisiae amino-terminal domain. Perhaps, like yeast mtRNA polymerase, the CRP1-like domain in vertebrate mtRNA polymerases may be involved in coupling RNA processing or translation activities to transcription. Experiments are currently in progress to determine whether, in fact, the amino-terminal extension of human mtRNA polymerase has a role similar to that provided by the yeast enzyme and to determine whether the amino acid similarity to CRP1 (i.e. the PPR motifs) is of functional and evolu-
tionary significance. Such experiments should lend new in-
sights into the regulation of human mitochondrial genome expression and replication and its impact on human disease.

Acknowledgments—We thank Yuan Wang for early contributions to this work, Melissa McKay for suggesting the PCR mutagenesis protocol, and Dr. Bonnie Seidel-Rogol for critical reading of the manuscript.

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