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The yeast nuclear gene *suv3* affecting mitochondrial post-transcriptional processes encodes a putative ATP-dependent RNA helicase

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ABSTRACT Mitochondrial gene expression is controlled largely through the action of products of the nuclear genome. The yeast nuclear gene *suv3* has been implicated in a variety of mitochondrial posttranscriptional processes and in translation and, thus, represents a key control element in nuclear–mitochondrial interactions. We have exploited a property of a mutant allele of *suv3*, *SUV3-1*, that causes, among other effects, a massive increase in the abundance of excised group I introns to clone the wild-type gene by a strategy of colony Northern hybridization. We have determined that the 84-kDa deduced protein product of the *suv3* gene, which maps to chromosome XVI, has a typical mitochondrial targeting presequence and additional sequence motifs that suggest that it belongs to a family of ATP-dependent RNA helicases, enzymes whose importance in post-transcriptional and translational events has recently become apparent. We have identified the *SUV3-1* mutation as a G → T transversion that creates a Val → Leu substitution in a 10-amino acid block that is highly conserved among ATP-dependent RNA helicases. We discuss some implications of this mutation on the effects of the *SUV3-1* allele on mitochondrial RNA metabolism.

The formation of the 3′ ends of most yeast mitochondrial mRNAs occurs by posttranscriptional processing at a highly conserved dodecamer sequence (1–3). The requirements for processing at a dodecamer to generate a functional mRNA became apparent with the discovery of a mutant, PZ206, in which the dodecamer and 195 base pairs (bp) of flanking 3′ noncoding sequences at the end of the *varl* gene were deleted (4, 5). This deletion resulted in the formation of *varl* transcripts with aberrant 3′ ends such that those RNAs were not functional for translation of the varl protein. Since varl is required for assembly of the 37S mitochondrial ribosomal subunit (6, 7), PZ206 cells are respiratory-deficient and thus unable to grow on glycerol.

This laboratory has described (8, 9) the properties of a dominant suppressor allele (*SUV3-1*) of a nuclear gene (*suv3*) that allows the aberrant *varl* transcripts in PZ206 to be translated, restoring respiratory function to those cells. In those studies, it was found, unexpectedly, that in cells containing a wild-type mitochondrial genome, the suppressor *SUV3-1* allele has pleiotropic effects on mitochondrial RNA metabolism. For example, there is a dramatic overaccumulation of the excised ω intron in *SUV3-1* cells and an overaccumulation of six of seven other excised group I introns tested; most of these other excised introns are undetectable in wild-type (*suv3*) cells. Analysis of mRNA abundance in *SUV3-1* cells shows that it also causes a 2- to 5-fold reduction in the mRNA levels of the *COB* gene, and a 20-fold reduction in the levels of COXI mRNA; the lowered abundance of the COXI mRNA is due to a decrease in the splicing of *COXI* intron 5β. Despite the reduction in the abundance of those mRNAs, protein labeling experiments indicate that the amounts of cytochrome *b* and coxI proteins are about the same as in wild-type cells. These varied effects of the *SUV3-1* suppressor allele in cells with a wild-type mitochondrial genome suggest that the wild-type *suv3* allele plays diverse and important roles in mitochondrial RNA metabolism and translation.

In this report, we describe the cloning and sequencing[‖] of the wild-type *suv3* allele by using a strategy of colony Northern hybridization (10). Our results show that *suv3* is essential for glycerol growth and is likely to encode a mitochondrial ATP-dependent RNA helicase, a family of enzymes that function in RNA metabolism and translation (11, 12). We show further that the suppressor *SUV3-1* allele contains a point mutation that results in a conservative amino acid change in a region of the protein that is highly conserved among ATP-dependent RNA helicases. We discuss the possibility that *SUV3-1* encodes an altered but functional RNA helicase that accounts for its pleiotropic effects in mitochondria.

MATERIALS AND METHODS

Strains and Growth Conditions. The basic yeast strains used in this study were α15B (*Mat*α *ura3 trp SUV3-1* $\rho^+\omega^+$) and BWG1 (*Mat*a *his134-519 ura3 ade1 leu2 suv3* $\rho^+\omega^+$). Cells were grown in medium containing 1% yeast extract, 1% Bacto Peptone, and 2% (wt/vol) dextrose (YPD).

Plasmids for Transformation and Disruptions. Plasmids used in this study were pYcP50 (13); pJVM-1URA3, an *ARS–CEN* shuttle vector kindly provided by J. Moran (University of Texas Southwestern Medical Center, Dallas); and pGEM3Zf(+) (Promega).

Colony Northern, Northern, and Southern Blotting Analyses and DNA Sequencing. For Northern blots, total cellular RNA (see Fig. 1) or total mitochondrial RNA (see Fig. 5) was isolated and fractionated on 1.2% agarose/formaldehyde gels. Colony Northern analyses using an ω intron probe {a 495-bp *Hin*dIII fragment of the 1.1-kilobase (kb) ω intron labeled by random priming with [α-^{32}P]dATP} were performed as described by Stepien and Butow (10). A wild-type yeast library in a pYcP50ARS-CEN vector was used to transform α15B $\rho^+\omega^+$ cells. Ura$^+$ transformants were grown overnight on Hybond-N filters (Amersham) on solid YPD. Yeast genomic DNA for Southern blot analysis was prepared as described by Parikh *et al.* (14). DNA sequencing of the wild-type *suv3* allele was carried out using the Sequenase

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[‖]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M91167).

system (United States Biochemical) with double-stranded DNA as template and 10 synthetic DNA primers.

Disruptions. *URA3* disruptions of *suv3* were made by transforming cells with linear DNAs representing the *Bam*HI-downstream *Nsi* I (d1) and *Eco*RI-downstream *Nsi* I (d2) fragments containing *URA3* inserts as shown in Fig. 2. The d1 disruption was constructed by placing a 1.6-kb *Pst* I–*Hpa* I *URA3* fragment into the upstream *Nsi* I and *Eco*RV sites of a 3.3-kb *Bam*HI downstream *Nsi* I *suv3* fragment in pGEM3Zf(+). The d2 disruption was constructed by placing a 1.6-kb *Hpa* I–*Kpn* I *URA3* fragment into the *Eco*RV and *Kpn* I sites of a 2.8-kb *Nsi* I *suv3* fragment in pGEM3Zf(+).

PCR Amplification. The oligonucleotides 5′-CCAAAATGCAGAAGCTTGATACTTTC-3′ and 5′-GTATCATGTTAAGCTTTCAAGCAGTG-3′ were synthesized to the 5′ and 3′ flanking sequences of *suv3*, respectively (where underlined sequences are *Hin*dIII sites). Each oligonucleotide contained a 1-bp mismatch from the corresponding template sequence to introduce the flanking *Hin*dIII sites in the amplified DNA to facilitate cloning into pGEM3Zf(+). PCR conditions were as recommended by the manufacturer (Perkin–Elmer/Cetus). Reaction mixtures were denatured at 94°C for 1 min, annealed at 54°C for 2 min, and polymerized at 72°C for 3.5 min.

RESULTS AND DISCUSSION

Cloning the *suv3* Allele. To make use of available wild-type yeast genomic libraries to clone the *suv3* allele, we took advantage of the observation that, in strains containing the ω intron in the mitochondrial 21S rRNA gene, the excised intron is 10- to 90-fold more abundant in *SUV3-1* suppressor strains than in wild-type cells (ref. 8 and Fig. 1*A*). Moreover, the *SUV3-1* allele shows a gene-dosage effect in that ω intron abundance in *suv3/SUV3-1* heterozygotes is greater than in *suv3* homozygous cells but less than in cells homozygous for *SUV3-1* (Fig. 1*A*). To screen for potential *suv3* transformants, we developed a semiquantitative yeast colony Northern hybridization procedure (10) (Fig. 1*B*) to monitor the level of ω intron RNA in haploid *SUV3-1* cells transformed with a recombinant library of wild-type yeast DNA, by reasoning that we could detect *suv3* transformants as a reduction in the ω signal intensity.

The *SUV3-1* haploid strain α15B was transformed with a wild-type *Saccharomyces cerevisiae* genomic library consisting of 15- to 25-kb inserts cloned into the *URA3* shuttle vector pYcP50 (13). Among 4200 Ura^+ transformants, we identified one colony of respiratory competent cells whose ω signal was close to that of a heterozygous *suv3/SUV3-1* diploid (Fig. 1*B*). The reduction in the ω signal in that transformant was dependent upon the presence of a plasmid (p38B47) containing a 22-kb insert, since spontaneous loss of that plasmid resulted in an increase in the ω intron RNA signal to the level of the original strain α15B (Fig. 1*B*).

Subcloning of the 22-kb insert in p38B47 into an *ARS–CEN URA* vector, pJVM-1, and transforming into α15B yielded a minimum 2.8-kb *Nsi* I fragment capable of reducing ω intron

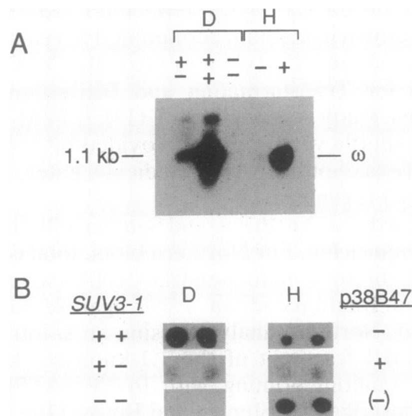
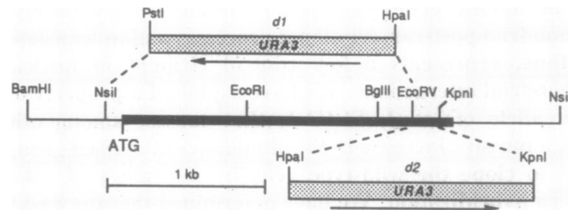


FIG. 1. Effect of *suv3* and *SUV3-1* alleles on ω intron RNA abundance. (*A*) Northern blot analysis of ω intron RNA levels in total RNA from diploid (lanes D) strains in a *suv3/SUV3-1* heterozygous configuration (+/−), homozygous for *SUV3-1* (+/+), and homozygous for *suv3* (−/−) and from haploid (lanes H) *suv3* (−) and *SUV3-1* (+) strains. (*B*) Colony Northern hybridization of ω intron RNA levels in the same diploid (D) strains noted in *A*. H is the ω intron RNA hybridization signal in a *SUV3-1* haploid (α15B) where − indicates before and + indicates after transformation with plasmid p38B47. (−) is the ω intron signal after spontaneous loss of the plasmid. Duplicate colony hybridizations are shown for each experiment.

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MALVKYSTVFFPLRSLRLFVSIKKAYYHSEPHSIDLFHDK   40

DWIVKRPKFLNLPKNEHSKLDIFQFNFNKSESNNVYLQDS   80

SFKDNLDKAMQFIYNDKLSSLDAKQVPIKNLAWLKLRDYI  120

YQQLKDPKLQAKTYVPSVSEIIHPSSPGNLISLLINCNKI  160

SNLVRKSVLKYSLSNNITTLDKFIHVLQQTFDHVYEQEIL  200

PMMTNTDDTDGAHNVDITNPAEWFPEARKIRRHIIMHIGP  240
                                  I
TNSGKTYRALQKLKSVDRGYYAGPLRLLAREVYDRFHAEK  280
                            Ia
IRCNLLTGEEVIRDLDDRGNSAGLTSGTVEMVPINQKFDV  320

VVLDEIQMMSDGDRGWAWTNALLGVVSKEVHLCGEKSVLP  360
    II
LVKSIVKMTGDKLTINEYERLGKLSVEEKPIKDGIKGLRK  400

GDCVVAFSKKKILDLKLKIEKDTNLKVAVIYGSLPPETRV  440
     IV
QQAALFNNGEYDIMVASDAIGMGLNLSIDRVVFTTNMKYN  480
                   V
GEELMEMTSSQIKQIGGRAGRFKSRSASGGVPQGFITSFE  520
                   VI
SKVLKSVRKAIEAPVEYLKTAVTWPTDEICAQLMTQFPPG  560

TPTSVLLQTISDELEKSSDNLFTLSDLKSKLKVIGLFDLM  600

EDIPFFDKLKLSNAPAKDMPMVTKAFTKFCETIAKRDTRG  640

LLSYRLPFNLLDYNCIPNESYSLDVYESLYNIITLYFWLS  680

NRYPNYFIDMESAKDLKYFCEMIIFEKLDRLKKNPYAHKP  720

FGSTRGHLSSSRRRLRT                        737
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FIG. 2. Deduced amino acid sequence (single letter code) of the *suv3* gene. The diagram shows the restriction map of a 3.3-kb fragment of yeast genomic DNA. Solid bar indicates the 2211-bp open reading frame of *suv3* beginning with an ATG as indicated. Two *URA3* disruptions of the open reading frame, d1 and d2, are shown. Arrows indicate the direction of transcription of *URA3*. The dotted line under the N-terminal portion of the sequence indicates a putative mitochondrial targeting signal; the solid lines indicate blocks of similarity to ATP-dependent RNA helicases as shown in Fig. 3.

RNA accumulation (Fig. 2). Nucleotide sequencing of this fragment revealed an open reading frame capable of encoding a protein of 737 amino acid residues. The result that the first 25 or so residues of the deduced protein sequence contained a distribution of hydroxylated and positively charged amino acids characteristic of mitochondrial presequence targeting signals in yeast (15) suggests that the product of this gene is likely to be a mitochondrial protein. A search of the available data bases (see below) did not reveal identity to any known protein. However, as described below, the deduced protein product of this gene contains a number of conserved amino acid motifs characteristic of ATP-dependent RNA helicases.

Genetic Analysis of *suv3* Disruptions. Two *URA3* disruptions of the *suv3* coding region were made in the ρ^+ haploid strain BWG1 as indicated in Fig. 2, one (d1) retaining ≈18% and the other (d2) retaining 82% of the *suv3* coding region. For both disruptions, which we have verified by Southern blot analysis (data not shown), cells are viable on glucose medium but cannot grow on nonfermentable carbon sources. Analysis of subclones from 29 independent transplacements representing both d1 and d2 disruptions reveals that these cells are converted to both ρ^- and ρ° petites (not shown). To identify the cloned gene as *suv3*, a heterozygous diploid was constructed between a BWG1 *suv3* ρ° strain carrying the d2 disruption and α15B *SUV3-1* $\rho^+\omega^+$ and then sporulated. Tetrad analysis showed that Ura^+ glycerol-negative spores cosegregated 2:2 and that the ura^- glycerol-positive (Gly^+) spores accumulated high levels of ω intron RNA typical of *SUV3-1* cells. In a second experiment, a *SUV3-1/suv3* diploid strain obtained from a cross between α15B *SUV3-1* $\rho^\circ\ \omega^+$ and BWG *suv3* ρ° was disrupted with *URA3* (d2, Fig. 2) and URA^+ Gly^+ cells were sporulated. Random spore analysis showed that all Gly^+ spores were ura^- and all had low levels of the ω intron typical of *suv3* cells. Thus these results show that the gene we cloned is *suv3* and that the mutant allele *SUV3-1* was disrupted in the diploid. Finally, our sequence analysis shows that *suv3* is immediately adjacent to the 5′ end of the *tsm0115* locus, which encodes acetoacetyl CoA thiolase, located on chromosome XVI (16).

***suv3* Encodes a Putative ATP-Dependent RNA Helicase.** To characterize the suv3 product further, a detailed search (February 3, 1992) of several protein sequence data bases (Protein Identification Resource, Version 30.0; SwissProt, Version 20.0; GenPept, Release 70.0; GenInfo Backbone Database) was carried out with the BLAST series of programs (17, 18). This analysis revealed that the suv3 product has very weak similarities, from residues 440 to 487, with only two proteins in the data bases, the putative *S. cerevisiae* ATP-dependent RNA helicases CA3 (19) and CA8/PRP28 (20). Further analysis with other software, such as MACAW (21), revealed similarities with many other RNA-dependent helicases in a number of regions of known conserved amino acid motifs identified by Gorbalenya *et al.* (22, 23) and Hodgman (24) including an NTP binding site, SGKT, in block I, a version of the "DEAD box" (11) in block II, and a putative RNA binding domain, GRAGR, in block VI (Fig. 3). However, by using the MACAW software to determine a *P* value, there is no statistically significant block III in the suv3 product: either it is deleted or the similarity in this region has become too redundant to observe. Finally, we note a cluster of basic amino acid residues at the very C terminus of the deduced suv3 protein.

The clustering of the similarity blocks among these helicases is not uniformly distributed along the length of the

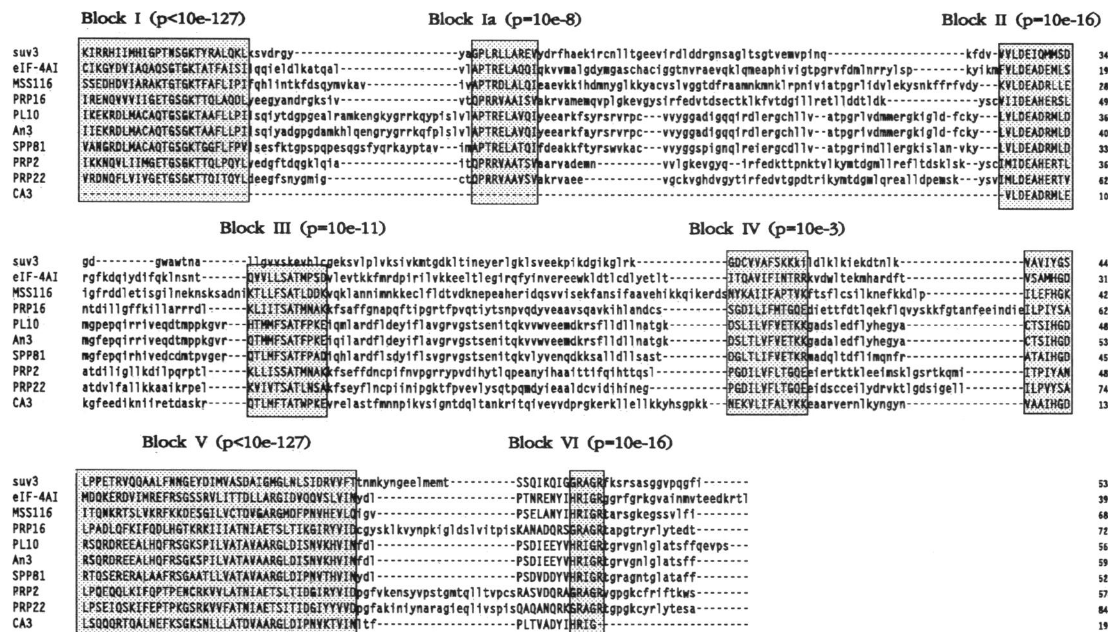

FIG. 3. Amino acid alignment of conserved domains of the deduced suv3 protein product. The central and C-terminal regions of the known similarities of several putative ATP-dependent RNA helicases were extracted from the protein data bases or the literature and were aligned with the analogous region of suv3 (i.e., from residue 239 to residue 531) by using the multiple sequence alignment program MACAW (19), which can locate, analyze, and assess the significance of these regions of sequence similarity. Gorbalenya *et al.* (22, 23) and Hodgman (24) have identified several regions of sequence similarity in these proteins. We have constructed an alignment similar to that of Gorbalenya *et al.* (22, 23) and have identified regions of statistically significant sequence similarity by using the same nomenclature. However, we find that suv3 contains only six of these blocks of similarity; block III seems to have become redundant or has been deleted. In addition, our alignment of block VI is slightly different and relies on the conservation of two arginine residues. The mean pair scores (21) for the alignment are 36.2, 21.7, 24.2, 21.6 (without suv3), 15.8, 73.3, and 27.8, and the *P* values are $<10^{-127}$, 5.8×10^{-8}, 5.6×10^{-16}, 1.5×10^{-11} (without suv3), 6.1×10^{-3}, $<10^{-127}$, and 2.2×10^{-16}, respectively, for blocks I, Ia, II, III, IV, V, and VI. Block V is the longest stretch of similarity consisting of 48 amino acids and contains the sequence in suv3 that was shown to be similar to CA3 and CA8 by using the BLAST programs. There is a variable distance between block V and block VI that contains either the GRAGR or the HRIGR sequence. These motifs are part of a larger block of sequence similarity in a stretch of 13 amino acids. The correlation between the motif in block VI and the DEA[DH] block (i.e., block II) has been mentioned (25).

proteins and thus it would be extremely difficult to write a "consensus" sequence for this class of proteins to query a data base for extraction of all potential helicases. A solution to this problem of identifying diagnostic motifs is to write the multiple alignments as a UNIX regular expression. We have written the following regular expression for ATP-dependent RNA/DNA helicases: [STVPM][GNQ][ASTGLV]GK[ST].*[AVIMF][VFMLI]DE[AI][DHQM].*[HG]R[AI][STAGVIL]R, where the period is any residue, the asterisk is any number of the preceding residue, and the brackets are used to enclose a group of residues, any one of which can be at that position in the sequence.

With this expression, a search of all the available protein sequence data bases listed above with an exact pattern-matching program called FPAT (C. F. Butler, National Institutes of Health, Bethesda, MD) results in >20 matches, all of which are helicases or putative helicases from a range of species including potyviruses, bacteria, and mammals. It is therefore possible to identify other proteins as presumptive helicases by using this regular expression and refinements thereof with any matching algorithm, although an absolute conservation of sequence motifs in all helicases is not required.

The *SUV3-1* Mutation. To determine the nature of the *SUV3-1* mutation, oligonucleotides to the 5' and 3' flanking regions of the *suv3* coding region were used to PCR amplify a 2.4-kb fragment of genomic DNA from the *SUV3-1* strain α15B in six reactions. Subsequent nucleotide sequence analysis of the independently amplified *SUV3-1* mutant allele reveals in each case a G → T transversion at position 814 in the coding sequence, one example of which is shown in Fig. 4; no other changes from the wild-type *suv3* allele were noted. The transversion changes Val-272 → Leu at the end of block Ia of the helicase domain (see Fig. 3).

To demonstrate that the G → T transversion mediates the observed SUV3-1 phenotype of overaccumulation of the excised ω intron, we replaced the *SUV3-1* T^{814} mutant allele in the BWG1 *suv3::URA3* (d2) ρ^0 strain with a linear 2.3-kb *Acc* I fragment; as a control, the same fragment was used from the *suv3* wild-type allele. Ura^- colonies were selected on plates containing 5-fluoroorotic acid, and a wild-type mitochondrial genome was introduced by cytoduction. Since the *suv3::URA3* disruptions are gly^-, any Gly^+ cytoductants (*SUV3-1* or *suv3*) will be candidates for transplacements. By using this approach, 10 Gly^+ ura^- colonies from the transformation with *SUV3-1* DNA and 7 Gly^+ ura^- colonies from the transformation with *suv3* DNA were isolated; these were first screened by colony hybridization for ω intron abundance. RNA from one positive clone from the *SUV3-1* transplacement, a negative clone, and one clone from the *suv3* transplacement were analyzed by Northern hybridization for ω intron abundance. (Since the G → T transversion is located 832 bp away from the 5' end of the *Acc* I site and 990 bp upstream of the start of the *URA3* disruption, transplacements with *SUV3-1* DNA can resolve on either side of the mutation, so that we would anticipate obtaining both mutant and wild-type alleles.) Fig. 5*A* shows the expected overaccumulation of the ω intron only in RNA from the positive *SUV3-1* transformant. Sequence analysis of the relevant region of the *SUV3-1–suv3* alleles (Fig. 5*B*) shows that only the allele from the strain that showed an increase in ω abundance contains the G → T transversion. These data confirm the Val-272 → Leu change in *SUV3-1*. Although the mutation in *SUV3-1* is a conservative substitution, we have noted that of 31 known or putative ATP-dependent RNA helicases whose sequences we have examined, 28 of them

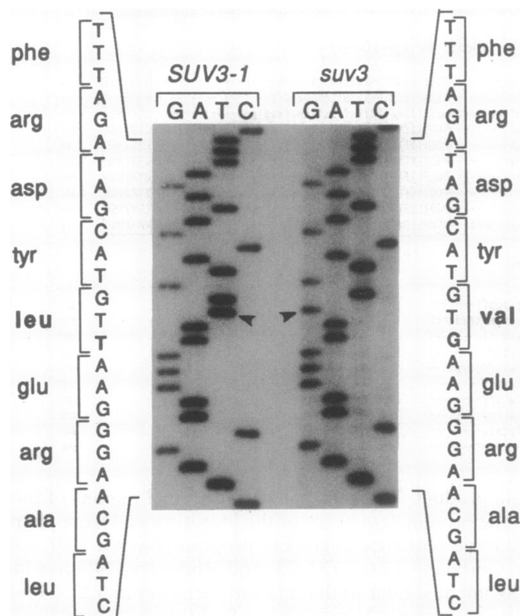

FIG. 4. *SUV3-1* contains a G → T transversion at position 814 of the coding region. Shown are the DNA sequence of the region of *SUV3-1* containing T^{814} from a representative of PCR-amplified *SUV3-1* DNA and the corresponding *suv3* sequence from the wild-type pYcP50 library. The deduced amino acid sequences are shown on each side of the figure.

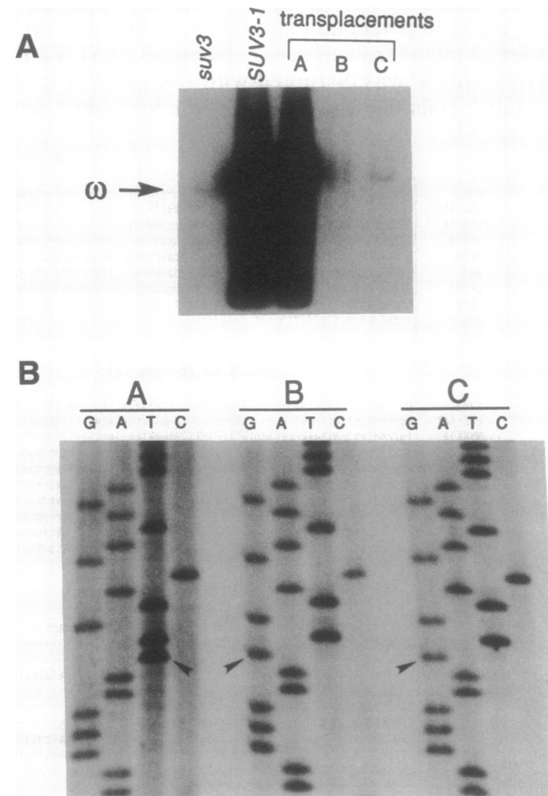

FIG. 5. Transplacement of T^{814} from *SUV3-1* into the *suv3* allele results in overaccumulation of the ω intron. (*A*) Northern blot analysis of ω intron abundance in RNA of three *suv3::URA3* (d2) transplacements: Lanes: A, RNA from cells transformed with *SUV3-1* DNA that scored positive for increased ω RNA in colony Northern hybridization; B, same as above except these cells scored negative for increased ω abundance; C, cells transformed with *suv3* DNA. RNA loads were normalized to the level of the 15S mitochondrial rRNA. (*B*) DNA sequence of the relevant region of *suv3–SUV3-1* DNA. Sequencing lanes A–C correspond to the cells from lanes A–C in *A*. The arrows indicate nucleotide 814 within the *suv3/SUV3-1* coding region.

contain a valine or isoleucine at that position in block Ia, whereas three others have a threonine there. Further studies will be required to determine how this substitution affects the putative helicase activity of the suv3 product.

RNA Helicase Functions in Mitochondrial RNA Metabolism. ATP-dependent RNA helicases identified biochemically or inferred from sequence information have been implicated or shown directly to be involved in translation and pre-mRNA splicing (12). For example, at least six such genes of the DEAD box family have been identified in yeast to function in nuclear pre-mRNA splicing (20, 25–29). Seraphin *et al.* (30) identified a nuclear gene of that family, *MSS116*, that appears to be required for splicing of several introns in the yeast mitochondrial genome. Although *MSS116* and *suv3* are distinct genes, mutants of both affect the excision of some of the same introns in the cytochrome *b* and *COXI* genes, e.g., bI3 and aI5β (9, 30). However, disruptions of *MSS116* do not result in loss or destabilization of the mitochondrial genome, whereas *suv3* disruptions lead to petite formation. The latter result would be expected if *suv3* were also required for one or more steps in mitochondrial translation, since it is well-established that mitochondrial protein synthesis is required for the maintenance of the ρ^+ mitochondrial genome (31, 32). This view is also supported by the effects of *SUV3-1* on the translation of aberrant var1 transcripts and apparent enhanced translational efficiency of the cytochrome *b* and COXI mRNAs (9). It is noteworthy in this connection that the *SB4* gene product of yeast (33), also a putative ATP-dependent RNA helicase, was identified as a suppressor allele of a block in translation.

Recycling of components of the splicing complex and release of excised introns has been suggested to be an important function of RNA helicases (20, 25, 28). Therefore, a plausible view of the effect of the *SUV3-1* allele on the overaccumulation of group I introns is that the helicase activity of its product is altered or compromised such that the excised introns are less efficiently released from splicing complexes in mutant cells than in wild-type cells. In *SUV3-1* cells, those introns might, therefore, be protected from nuclease attack, leading to their overaccumulation. Consistent with this view is the observation that cleavage of the dodecamer located within the ω intron, unlike other dodecamers tested, is blocked in *SUV3-1* cells, which suggests that the dodecamer sequence is not accessible to a dodecamer cleaving activity (3).

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