N2,N2-Dimethylguanosine-specific tRNA Methyltransferase Contains Both Nuclear and Mitochondrial Targeting Signals in Saccharomyces Cerevisiae

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Abstract. The TRM1 gene of Saccharomyces cerevisiae encodes a tRNA modification enzyme, N2,N2-dimethylguanosine-specific tRNA methyltransferase, which modifies both mitochondrial and cytoplasmic tRNAs. The enzyme is targeted to mitochondria for the modification of mitochondrial tRNAs. Cellular fractionation and indirect immunofluorescence studies reported here demonstrate that this enzyme is also localized to the nucleus. Further, immunofluorescence experiments using strains that overproduce the enzyme show a staining at the periphery of the nucleus suggesting that the enzyme is found in a subnuclear destination near or at the nuclear membrane. There is no obvious cytoplasmic staining in these overproducing strains. Fusion protein technology was used to begin to localize sequences involved in the nuclear targeting of this enzyme. Indirect immunofluorescence studies indicate that sequences between the first 70 and 213 NH2-terminal amino acids of the methyltransferase are sufficient to target Escherichia coli β-galactosidase to nuclei.

Primary transcripts of tRNA genes undergo diverse posttranscriptional reactions during their maturation. A large proportion of these processing steps result in the modification of specific nucleoside residues with the majority of the modifications being methylation reactions. Although the biological roles of these methylations are not clearly defined, the methylated nucleosides account for 30-70% of the modified nucleosides in tRNA (Nau, 1976). Mitochondrial and cytoplasmic tRNAs have different but overlapping populations of methylated bases (Martin et al., 1976). Therefore, enzymes that are required for the synthesis of the methylations unique to each type of cellular RNA are likely specifically targeted to either mitochondria or the nucleus/cytoplasm to function. Enzymes responsible for the synthesis of the same modified bases in both mitochondrial and cytoplasmic tRNAs could either be functionally related but structurally distinct isozymes or they could be structurally identical but found in multiple cellular locations.

trm1 is a nuclear mutation that affects N2,N2-dimethylguanosine (m2G) biosynthesis of both mitochondrial and cytoplasmic tRNAs in Saccharomyces cerevisiae (Hopper et al., 1982). Strains carrying the mutation and thus unable to make m2G do not show any noticeable phenotype. We have previously reported the isolation of the TRM1 gene and demonstrated that it encodes m2G-specific tRNA methyltransferase (Ellis et al., 1986). Two forms of the enzyme can be made from this gene because some mRNAs transcribed from this gene include two AUGs separated by 15 codons while other, shorter mRNAs have only the second AUG from which to initiate protein synthesis (Ellis et al., 1987). Enzyme synthesized from either the first or second AUG can be imported into mitochondria for m2G base modification. The m2G base modification of cytoplasmic tRNAs appears to be carried out largely by enzyme synthesized from the second AUG (Ellis et al., 1987).

Methylation of nucleosides represents a major modification step in tRNA maturation. Although at least eight distinct modification enzymes have been identified in S. cerevisiae, very little is known about the intracellular locations of these enzymes (Bjork and Svensson, 1969). The fact that some mitochondrial tRNAs contain mG suggests that the enzyme must be found in mitochondria, a supposition supported by the identification of NH2-terminal sequences that are sufficient to target passenger proteins to mitochondria (Ellis et al., 1989). Biochemical evidence indicates that nuclear precursor tRNAs contain mG, and they are believed to be nuclear restricted (Peebles et al., 1979; Nishikura and De Robertis, 1981). This suggests that the enzyme responsible for this modification must be imported into the nucleus. It was not known whether the enzyme is also located in the cytoplasm.

Cell fractionation and indirect immunofluorescence experiments demonstrate that mG-specific tRNA methyltransferase is a nuclear enzyme in yeast. Specific nuclear rim staining is observed in strains that overproduce the enzyme, suggesting that the enzyme is at special subnuclear locations, possibly on or close to the nuclear membrane. Gene fusion

1. Abbreviations used in this paper: β-gal, β-galactosidase; DAPI, 4',6'-diamidino-2-phenylindole; mG, N2,N2-dimethylguanosine.

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technology has shown that the NH2-terminal 213 amino acids of the enzyme contain sufficient information to target β-galactosidase (β-gal) to both the nucleus and mitochondria. Since a fusion protein containing only the NH2-terminal 70 amino acids of the modification enzyme is not targeted to nuclei, we infer that the region between amino acid 70 and 213 of this enzyme contains sufficient information to target β-gal to the yeast nucleus and have identified a stretch of amino acids in this region that are similar to other known nuclear targeting signals.

**Materials and Methods**

**Strains and Media**

Strains used in these experiments were MH41-7B (MATa, ade2, his3), W303-1b (MATa, ade2-1, his3-11/15, leu2-3/112, ura3-1, trpl-1, can1-100), SN1015-2a (MATa, SUP4, trm1, trm2, gal1, gal7, ade2-1, leu1, ura3, met1, his3), DB745 (MATa, ura3-52, leu2-3/112, suc2-9, his4-519, gal2).

**Plasmids**

HC-3 is a Yep24-TRM1 recombinant plasmid which supports the synthesis of two forms of mG-specific tRNA methyltransferase differing by 16 NH2-terminal amino acids because two in-frame ATGs are present in the gene and both are used (Ellis et al., 1986). A TRM1 gene, altered by site-directed mutagenesis to introduce a Hind III site at position 8, was cloned into the Hind III site of plasmid pMB272 which contains the Galt-Gall10 promoter region (Johnston and Davis, 1984). This first ATG is upstream of the Hind III site so that synthesis of mG-specific tRNA methyltransferase is from only the second ATG of the wild-type TRM1 gene in the resulting plasmid, pGT554. TRM1-LacZ fusion genes were produced by cloning an Eco RI/Bam HI fragment containing the TRM1 promoter, both in-frame ATGs, and 213 codons from the first ATG (Ellis et al., 1987) into the Eco RI/Bam HI sites of the vector, pSEY101 (Emr et al., 1986). The resulting plasmid, pTRMZ213, generates chimeric proteins 1-213 TRM1 and 17-213 TRM1 derived from the first or second in-frame ATG, respectively. Both have β-gal activity. Plasmid pTRMZ70 was derived from pTRMZ213 by deleting a Bgl II/Bam HI fragment. This deleted TRM1 sequence coding for amino acids 71 to 213 and thus the plasmid generates chimeric proteins 1-70 TRM1 and 17-70 TRM1 with β-gal activity but containing fewer amino acids derived from the TRM1 sequence than those proteins derived from pTRMZ213. An identical strategy was used to construct plasmids that would synthesize only 17-213 TRM1 and 17-70 TRM1 proteins by using a TRM1 gene in which the first ATG had been altered by site-directed mutagenesis (Ellis et al., 1987). The plasmid pLG669Z produces authentic β-gal (Guarente et al., 1982). All plasmids were introduced into yeast by the method of Ito et al. (1983). DNAs from yeast transformants were prepared using the procedure developed by Zakian and Scott (1982) and confirmed either by restriction analysis or by DNA sequencing (Sanger et al., 1977).

**Preparation of Crude Extracts for the mG-specific tRNA Methyltransferase Assay and Western Blot**

Yeast strain DBF45 carrying pGT554 was grown in either YPD(dextrose) or YPGD(galactose) medium. Other strains were grown in media minus uracil (Hopper et al., 1982) and extracts prepared as described by Silver et al. (1984). Briefly, when the optical density of the culture reached 1.0 OD600, cells were harvested by centrifugation, washed with NET-NP(150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.4, 0.5% NP-40), and suspended in NET-NP buffer containing 1 mM PMSF. Glass beads were added to the suspension and mixed on a vortex mixer at maximum speed for five 30-s intervals separated by cooling in ice. After centrifugation at 15,000 g for 1 min, the supernatant was saved. Protein concentrations were determined using the method of Bradford (1976). mG-specific tRNA methyltransferase activity was measured as described previously (Ellis et al., 1986). Western blots were done according to the procedure described by Tobin et al. (1979).
and suspended in 8 ml of 1 M sorbitol, 20 mM K$_2$HPO$_4$-KH$_2$PO$_4$, pH 6.5, 0.5 mM CaCl$_2$, 0.5% 2-mercaptoethanol, 0.5 mM PMSF. Zymolyase 60000 was added to a final concentration of 0.25 mg/ml, and incubated at room temperature until spheroplast formation occurred. Spheroplasts were spun down at 3500 g for 10 min, and suspended in 2 ml lysis buffer containing 0.2% NP-40, 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 5 mM MgCl$_2$, 0.5 mM CaCl$_2$, 0.5 mM PMSF. 15 strokes in a Dounce homogenizer (Kontes Glass Co., Vineland, NJ) were used to lyse the spheroplasts.

Crude nuclei were collected by centrifugation at 3000 g for 5 min and suspended in 30 ml of 50% (wt/vol) percoll, 40 mM Pipes, pH 6.8, 10 mM MgCl$_2$, 0.5 mM PMSF, 0.05% Triton X-100. The suspension was then centrifuged at 21000 g for 35 min in a Ti-30 rotor. The nuclear band was harvested and the nuclei pelleted at 5000 g after diluting the percoll solution 10-fold with 0.1 M K$_2$HPO$_4$-KH$_2$PO$_4$, pH 6.5.

β-Galactosidase Assay

Assays for β-galactosidase were done according to Miller's procedure (Miller, 1972) with some modifications specific for yeast. 0.1-1 ml of yeast culture with an optical density of 1 OD$_{600}$ was mixed with 2 vol of Z buffer containing 0.1 M K$_2$HPO$_4$-KH$_2$PO$_4$, pH 7.0, 0.01 M KCl, 1 mM MgSO$_4$, and 0.05 M 2-mercaptoethanol. The mixture was centrifuged in a clinical centrifuge for 1 min. The cell pellet was suspended in 1 ml Z buffer. 50 μl CHCl$_3$ and 20 μl 0.1% SDS were added. The mixture was mixed for 10 s at maximum speed on a vortex mixer to break the cells. The cell suspension was then incubated at 28°C for 2 min. The enzyme substrate o-nitrophenyl-β-d-galactoside was prewarmed at 28°C. The enzyme reaction was started by adding 0.2 ml o-nitrophenyl-β-d-galactoside and continued till the reaction solution turned to light yellow. 0.5 ml of 1 M Na$_2$CO$_3$ was then added to stop the reaction. Cell debris and CHCl$_3$ were separated from the reaction solution by centrifugation. The top clear yellow solution was transferred to a new tube and the optical density (OD$_{420}$) was measured with a spectrophotometer.

Antimycin A-insensitive NADH-Cytochrome C Reductase Assay

The enzyme assay was done according to Sottocasa's procedure (Sottocasa et al., 1967). The blank was a mixture of 0.4 ml cytochrome C (0.1 mM), 0.1 ml KCN (100 mM), 0.1 ml antimycin A (0.5 mg/ml ethanol), 0.5 ml buffer (0.1 M K$_2$HPO$_4$-KH$_2$PO$_4$, 10$^{-4}$ M EDTA, pH 7.6) and either 10 μl mitochondrial suspension or 100 μl cytosol fraction. The blank was monitored for 1 min for the absorption change. In the assay, everything was the same except that 0.1 ml NADH (1 mM) was used and 0.4 ml, instead of 0.5 ml, buffer was added. The kinetic reaction was measured for 1 min.

Alcohol Dehydrogenase Assay

The assay was used as an assay for cytoplasmic contamination. This assay is based on the increase in absorbance at 340 nm which results from the reduction of NAD in the presence of alcohol and alcohol dehydrogenase (Valle and Hoch, 1955). The 10× reaction mixture contains 160 mM sodium pyrophosphate, pH 8.8, 3.3 M ethanol, and 83.3 mM NAD. Cell extracts and water were added to the reaction mixture to a final volume of 3 ml. The reaction was carried out for 5 min at room temperature and the optical density (OD$_{340}$) was measured with a spectrophotometer.

RNA Polymerase Assay

RNA polymerase was used as a nuclear marker. The assay was done according to the procedure of Winkley et al. (1985). The reaction mixture contains 60 mM Tris-HCl, pH 7.9, 2 mM MgCl$_2$, 500 μM UTP, CTP, and GTP, 10 μl α$^{32}$P]ATP (800 Ci/mmole), 50 mM (NH$_4$)$_2$SO$_4$, 150 μg/ml calf thymus DNA and cell extracts to final volume of 40 μl. The reaction was carried out at 30°C for 10 min, and stopped by spotting 20 μl of the reaction mixture onto DE81 paper. The remaining steps were based on Roeder's procedure (Roeder, 1974). DE81 filters were immersed in 0.5 M Na$_2$HPO$_4$, washed six times with the same buffer, then washed twice in water, twice in 95% ethanol, and once in ether. The filters were dried and radioactivity was measured in a scintillation counter.

Indirect Immunofluorescence

Indirect immunofluorescence experiments were carried out according to Kilmartin's procedure with some modifications (Kilmartin et al., 1982). 10 ml of a culture in early log phase were harvested in a clinical centrifuge for 5 min at maximum speed. Cell pellets were suspended in 5 ml of solution A containing 40 mM K$_2$HPO$_4$-KH$_2$PO$_4$, pH 6.5, and 80 mM MgCl$_2$, 0.6% formaldehyde was added to the suspension to fix cells at room temperature for 2 h without shaking. The fixed cells were spun down and washed three times with solution B containing 40 mM K$_2$HPO$_4$-KH$_2$PO$_4$, pH 6.5, 80 mM MgCl$_2$, and 1.2 M sorbitol. Cells were suspended in 1 ml solution B. 55 μl Glusulase, 10 μl β-mercaptoethanol, and 20 μl Zymolyase 100T (1 μg/ml in water) were added and the mixture was incubated at 37°C for 1 h to make spheroplasts.

Wells on slides were coated with 0.1% polylysine 400,000 by adding 10 μl of polylysine into each well and removing it by aspiration 10 s later. After the wells dried, they were washed three times with water. Spheroplasts were washed once with solution B and then suspended in 1 ml solution B. 10-μl spheroplasts were applied into each well and incubated for 20 s. The cell suspension was aspirated and the wells were air dried. 10 μl affinity purified antipeptide antibody diluted 1:10 to 1:50 was applied to each well, and incubated for 1.5 h at room temperature in a moist environment. The primary antibody was washed off with eight changes of solution F containing 0.73 mM KH$_2$PO$_4$, 145 mM NaCl, and 0.1% BSA. 10 μl of FITC-conjugated second antibody from 1:600 to 1:1000 dilution was added to each well and incubated for another 1.5 h at room temperature in a dark and moist environment. Solution F was again changed eight times to remove the secondary antibody. For double staining, 20 μl 4',6'-diamidino-2-phenylindole (DAPI, 1 μg/ml) was applied to each well and incubated for 1 min. The solution was removed and wells were washed once with PBS. Wells were mounted with mounting medium and the coverslip was sealed with nail polish. The results were analyzed with a Nikon Inc. Biological Microscope OPTIPHOT (Garden City, NY).

The primary antibody was the affinity-purified antipeptide antibody. The secondary antibody was goat anti-rabbit IgG, conjugated with FITC (Cappel Laboratories, Cochranville, PA). Nuclear and mitochondrial DNAs were stained by DAPI to serve as location markers for both the nucleus and the mitochondria. Anti-β-galactosidase antibody was purchased from Worthington Biochemical Corp. (Freehold, NJ) and purified through a β-gal-Sepharose 4B affinity column.

Results

The TRM1 Gene Encodes the m1G-specific tRNA Methyltransferase

We have shown previously that introduction of the TRM1 gene into trml mutant yeast cells restores the ability of the mutant to make m1G. Furthermore, expression of the TRM1 gene in Escherichia coli converts that organism into one that is able to make this particular base modification (Ellis et al., 1986). These experiments led us to propose that TRM1 encodes m1G-specific tRNA methyltransferase. Here we present two additional experiments that strengthen that conclusion. First, manipulations which alter the expression of the TRM1 gene in yeast alter the level of m1G-specific tRNA methyltransferase. Specifically, the TRM1 gene was introduced into a multicopy plasmid and placed under control of the inducible GALI promoter. This promoter drives increased transcription when yeast cells are grown in galactose. Cells containing the TRM1 gene under GALI control show a 30-fold induction of this specific methyltransferase enzyme activity above the noninduced level found in cells grown in glucose (Fig. 1).

A more direct demonstration that the TRM1 gene codes for the enzyme was obtained by using an antibody raised against a synthetic antigen whose sequence was predicted from the TRM1 open reading frame (Ellis et al., 1987). The TRM1 open reading frame predicts a protein of 63 kD and the antipeptide antibody recognizes a protein of 63 kD (Fig. 2 A, lane I). The smaller protein seen at 60 kD in this particular

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Figure 1. Alteration of TRM1 gene expression alters m\(^\ddagger\)G-specific tRNA methyltransferase activity. Methytransferase activity was measured in extracts prepared from cells containing the vector alone and grown in galactose medium (pBM272-gal), or from cells containing the pGT554 plasmid derived from pBM272 by cloning the TRM1 gene under control of the G\(\alpha\)l promoter and grown in glucose (pGT554-glu) or galactose (pGT554-gal).

The sample is presumably a proteolytic fragment of the 63 kD protein as it is not observed in most other preparations. For example, it is absent from Fig. 2 A, lane 2, and B, lane 1. The protein in Fig. 2 A, lane 2, was isolated from the original trm1 mutant while that in Fig. 2 A, lane 3, was from a strain of yeast carrying a disrupted allele of the TRM1 gene. No 63-kD protein was detected in this strain. The protein in lane 1 of Fig. 2 B was isolated from cells that produce the TRM1 product under the control of the GAL1 promoter. Wild-type cells transformed with the GAL1 promoter plasmid without the TRM1 gene make only wild-type levels of m\(^\ddagger\)G-specific tRNA methyltransferase (detected only on longer exposures of the Western blot) compared to cells carrying the TRM1 gene on the same vector. This result is consistent with the data on the enzyme activity present in Fig. 1. We conclude from these experiments that this antibody is specific to the TRM1 gene product.

To determine if the protein coded by the TRM1 gene is the m\(^\ddagger\)G-specific tRNA methyltransferase, enzyme assays were performed in the presence and absence of the antibody. When enzyme extracts were incubated with the antipeptide antibody before assaying for enzyme activity, enzyme activity was inhibited (Fig. 3). The total amount of protein was kept constant in each assay, but the ratio of immune to preimmune IgG was increased. Therefore, the increasing inhibition of the enzyme activity is specifically caused by an increasing amount of the antipeptide antibody used in each assay mixture. The assay was an inhibition assay, not a precipitation assay so the fact that the inhibition never reached 100% may be explained if interaction of the antibody with the enzyme did not cause 100% inhibition of activity.

The m\(^\ddagger\)G-specific tRNA Methyltransferase Is Located in the Nucleus

The m\(^\ddagger\)G-specific tRNA methyltransferase is a mitochondrial enzyme responsible for the modification of tRNAs encoded by mitochondrial DNA (Ellis et al., 1987). However, the cellular location for the addition of m\(^\ddagger\)G to cytoplasmic tRNAs was not known. To determine whether the methyltransferase is located in nuclei, cytoplasm, or both, cell fractionation

![Figure 2](image.png)

Figure 2. Identification of the TRM1 protein with immunoblots using the antipeptide antibody (A). Lane 1, the wild-type strain W303-1b; lane 2, the trm1 mutant strain SN1015-2a; and lane 3, the strain W303-IBTHT which carries a disrupted allele of TRM1. Overproduction of the TRM1 product from the GAL1 promoter (B). Lane 1, cells carrying TRM1 on a multicopy plasmid under the control of the GAL1 promoter; lane 2, wild-type cells transformed with the GAL1 promoter plasmid without the TRM1 gene. Upon longer exposure, TRM1 product is observed in B, lane 2. 100 μg protein was separated on a 10% SDS-PAGE and transferred to nitrocellulose. The primary antibody was affinity-purified antipeptide antibody and the secondary antibody was affinity-purified goat anti-rabbit antibody conjugated with alkaline phosphatase.

![Table 1](image.png)

Table 1. Enzyme Distribution between the Nucleus and Cytoplasm

| Enzyme          | Nucleus | Cytoplasm |
|-----------------|---------|-----------|
| β-galactosidase | 3       | 97        |
| ADHase*         | 11      | 89        |
| RNA polymerase  | 86      | 14        |
| m\(^\ddagger\)Gase† | 84      | 16        |

* Alcohol dehydrogenase.
† m\(^\ddagger\)G-specific tRNA methyltransferase.
studies were carried out. Yeast nuclei were isolated from wild-type cells by differential centrifugation and purified on a 50% percoll gradient. As shown in Table I, 86% of the RNA polymerase activity was associated with the isolated nuclei and 14% of this marker enzyme was not nuclear associated. Some of the activity in the nonnuclear fractions may arise from the mitochondrial-specific polymerase and some from leakage from nuclei. Cytoplasmic contamination to the nuclear fraction was assessed by assaying two nonnuclear activities. One is endogenous alcohol dehydrogenase activity, the other bacterial β-gal activity produced from an E. coli gene. This latter protein has been shown to be cytoplasmic (Douglas et al., 1984). Only 3% of the authentic β-gal and 11% of the alcohol dehydrogenase were associated with the isolated nuclei. In the same fractions, 84% of the m1G-specific tRNA methyltransferase activity from wild-type cells was associated with the nuclear fraction.

In addition to the cell fractionation studies, we carried out indirect immunofluorescence experiments using the antipeptide antibody. We found that the amount of enzyme in wild-type cells was below the limit of detection.

Fig. 4 shows micrographs of cells carrying the plasmid HC-3 which carries the wild-type TRM1 gene and thus produces protein from both ATGs. Ellis et al. (1986) showed that these cells produce 10 times more methyltransferase than do wild-type cells and that the vast majority of the protein is made from the second ATG. Fig. 4 a shows a micrograph of these cells stained with DAPI, a DNA-specific fluorescent dye. Fig. 4 b shows the staining pattern observed when these cells were treated with the antipeptide antibody followed by FITC-conjugated goat anti-rabbit IgG interacting with the antipeptide antibody. Bar, 10 μm.
Figure 5. Localization of the TRM1-β-gal fusion proteins by indirect immunofluorescence. Cells containing either pTRMZ213 (a and b) or pTRMZ70 (c and d) were prepared for indirect immunofluorescence and treated with affinity-purified rabbit anti-β-gal antibody, followed by FITC-conjugated anti-rabbit IgG to identify the fusion proteins (a and c). DAPI was used to stain both nuclear and mitochondrial DNAs (b and d). Note the absence of mitochondrial DNA in cells carrying pTRMZ70. Bar, 20 μm.

The NH$_2$-terminal 213 Amino Acids of the Enzyme Are Sufficient to Target β-Gal to Both Nuclei and Mitochondria

The TRM1 gene has two in-frame ATGs that are both used for the initiation of protein synthesis. The vast majority of the TRM1 gene products start from the second ATG and are used to modify both the mitochondrial and cytoplasmic tRNAs in wild-type cells (Ellis et al., 1987). To begin to determine what sequences within the enzyme can promote the proper intracellular localization of the protein, we created
several TRM1–LacZ fusion genes. The TRM1 gene with its normal promoter was fused to LacZ so proteins starting from the first ([1–213 TRM1]β-gal) or the second ([17–213 TRM1]β-gal) ATG of the gene can be made. A TRM1 gene that had the first ATG removed was also fused to LacZ so that only the [17–213 TRM1]β-gal fusion protein would be made. The results obtained with both constructions were the same, indicating that the vast majority of the staining is due to proteins beginning at the second ATG. Fig. 5 b shows the DNA staining pattern and Fig. 5 a shows the FITC staining pattern observed in cells containing the fusion gene with both ATGs. In this figure, the DAPI and FITC staining appear as dark signals against a light background. The protein produced by this gene is clearly targeted to both nuclei and mitochondria.

Genes that produce fusion proteins with fewer amino acids derived from the TRM1 gene were derived from those described above. These genes both produced a protein, [17–70 TRM1]β-gal, from the second ATG of the TRM1 gene and the gene that retained the first ATG also produces a longer protein, [1–70 TRM1]β-gal. β-gal activity in these cells is twofold more than those carrying the 213 amino acid fusion. As can be seen from comparing Fig. 5, c and d, these proteins fail to localize β-gal to the nucleus. Instead, what β-gal can be seen appears to be in a few discrete cytoplasmic regions. Since cells carrying these fusions have lost their mitochondrial DNA, and we have not been successful in identifying another mitochondrial marker in our studies, we cannot determine whether these regions of staining colocalize with mitochondria. On the other hand, the fact that the fusion, but not β-gal alone, causes loss of mitochondrial DNA suggests that the protein is being delivered to the mitochondria where it interferes in some way with the biogenesis of a functional organelle.

**Discussion**

Both mitochondrial and cytoplasmic tRNAs in *S. cerevisiae* contain m7G and a mutation in the TRM1 gene abolishes this modification in both tRNA populations. Evidence from expression studies of TRM1 as well as antibody inhibition experiments demonstrate that TRM1 encodes m7G-specific tRNA methyltransferase, the enzyme responsible for the m7G modification. The original trm1 mutant makes a 63-kD protein so that a missense mutation or small in-frame deletion must be responsible for the lack of enzyme activity in this strain. Cell fractionation and indirect immunofluorescence studies demonstrate that m7G-specific tRNA methyltransferase is a nuclear, not a cytoplasmic, enzyme. A nuclear location for this enzyme is consistent with the previous report that precursor tRNAs have m7G and are presumably modified inside the nucleus (Peebles et al., 1979; Nishikura and De Robertis, 1981). These previous experiments did not give insight as to whether some enzymes would also be found in the cytoplasm. In fact, our results suggest that the enzyme is predominantly in the nuclei and mitochondria.

How a protein with two different destinations is sorted in the cell is unknown. The observation that the fusion protein 17–213 TRM1 β-gal is localized to mitochondria and nuclei suggests, but does not prove, that sequences important for the targeting of the native TRM1 product to dual compartments may be located in this region of the protein. Ellis et al. (1989) have constructed fusion proteins between the amino-terminal sequences of TRM1 and cytochrome oxidase subunit IV or dihydrofolate reductase. They found that amino acids 1–48 and 17–48 coded by the TRM1 open reading frame were sufficient to target these passenger proteins to the mitochondria and that the longer TRM1 sequence resulted in more efficient import of the fusion proteins. We have not attempted to measure the relative efficiency of mitochondrial import of the TRM1–β-gal fusions and, indeed, have not even demonstrated convincingly that the [1–70 TRM1]/[17–70 TRM1]β-gal fusions are imported in vivo. Immunofluorescence staining appears concentrated in a few distinct regions of the cytoplasm but since we have never recovered cells that contain both the plasmid which directs the synthesis of these fusions and mitochondrial DNA, we can no longer use DAPI staining as a measure of colocalization. Results of cell fractionation studies show that these fusions do not accumulate in the mitochondria (data not shown). The loss of mitochondrial DNA caused by the fusions may be due to a secondary consequence of incomplete translocation of TRM1–LacZ fusion proteins such that the import of other nuclear-coded mitochondrial proteins needed for replication, stability, or segregation of mitochondrial DNA would be blocked.

The shorter fusions do not localize to nuclei. If there is not a dramatic difference in the stability of the different fusions then the region between amino acids 70 and 213 of TRM1 should contain information sufficient to target β-gal to nuclei. When comparing this region with known nuclear targeting signals of other nuclear proteins, we find a similarity between an amino acid cluster of nine amino acids within this region and one of the nuclear targeting signals of the glucocorticoid receptor and the nuclear targeting signal of SV40 large T antigen (Picard and Yamamoto, 1987; Kaldor et al., 1984a,b) as well as histone H2B (Moreland et al., 1987). In fact, seven out of nine amino acids are conserved between the m7G-specific tRNA methyltransferase and part of the nuclear targeting signal from the glucocorticoid receptor (Table II). We have initiated site-directed mutagenesis experiments to test the hypothesis that this sequence is important to the nuclear localization of native methyltransferase.

The m7G-specific tRNA methyltransferase produced in cells carrying the TRM1 gene on multicopy plasmids appears to be concentrated near or at the nuclear membrane. The indirect immunofluorescent staining pattern we observe is different from the patch-like pore staining and diffuse nucleoplasm staining reported for yeast tRNA ligase (Clark and Abelson, 1987) and the nucleoplasm staining of the yeast RNAII gene product (Chang et al., 1988). A concentration of staining is observed ~100–300 nm from the nuclear envelope for these proteins by EM techniques. The staining pattern of the yeast NSPI gene, at or near the nuclear membrane.

| Targeting Signal | Sequence |
|------------------|----------|
| SV-40 large T antigen* | Pro Pro Lys Lys Lys Arg Lys12 |
| Glucocorticoid receptor* | Arg Lys Thr Lys Lys Lys Lys17 |
| Histone H2B* | Lys Arg Lys Ser Lys Ala Lys |
| Methyltransferase | Lys Ser Lys Lys Lys Arg Cys102 |

* Known nuclear targeting signals.
(Hurt, 1988), is dot-like while that observed with TRMI is uniform. Recently, Adachi and Yanagida (1989) have observed a staining pattern quite similar to that we see with TRMI antibodies using antibodies to the crml" (chromosomal region maintenance) gene product of S. pombe. We do not know, of course, whether the mg-specific tRNA methyltransferase in wild-type cells localizes to the periphery of the nucleus because the level of protein has, so far, precluded our detection by the immunofluorescent technique. The question of the role that overproduction may play in the subnuclear localization we observe cannot be ignored. However, it seems less likely to us that overproduction of a protein would convert it from a nonperipheral to a peripheral protein. Overproduction of a known peripheral protein, the tRNA ligase in yeast, does result in an increase in nuclear staining away from the periphery as if sites near the membrane become saturated (Clark and Abelson, 1987). The fact that the [17–213 TRMI]-gal fusion protein has a diffuse location throughout the nucleus and its synthesis is driven by the wild-type TRMI promoter might argue against these ideas on the effects of overproduction. On the other hand, if sequences to the carboxy-terminal side of amino acid 213 are needed for subnuclear localization of native TRMI, they would be missing from this fusion. That sequences direct the subnuclear location of nuclear proteins has been demonstrated by Siomi et al. (1988). The first 20 amino acids of human T cell leukemia virus type I p27 are sufficient to localize β-gal in the nucleus. This region must, by itself, constitute a structure that is recognized by the nuclear transport machinery and that once imported interacts with nuclear components. Longer TRMI–LacZ fusions that could be useful in locating a subnuclear localization signal are currently being constructed.

Although there are quite a few proteins now identified that are shared by the mitochondria and the cytoplasm (Beltzer et al., 1986; Natsoulis et al., 1986; Wu and Tzagoloff, 1987; Chatton et al., 1988; Gillman, E. C., L. Slusher, N. C. Martin, and A. K. Hopper, unpublished observations), mg-specific tRNA methyltransferase is the only one to our knowledge that is shared by the mitochondria and the nucleus. As such, it presents a unique opportunity to ask how a single protein interacts with both compartments. In addition, while the TRMI gene is now well characterized, little is known about the function of mg modification on tRNAs and why the enzyme responsible for this base modification is located in the nucleus. Further analyses promise to increase our knowledge about both of these questions.

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