Molecular Biology of the Cell
Vol. 5, 1253–1263, November 1994

Nuclear mRNA Accumulation Causes Nucleolar Fragmentation in Yeast *mtr2* Mutant

Tatsuhiko Kadowaki,* Midori Hitomi, Shaoping Chen, and Alan M. Tartakoff*

Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106

Submitted July 29, 1994; Accepted September 26, 1994
Monitoring Editor: Randy W. Schekman

We have identified a set of genes that affect mRNA transport (*mtr*) from the nucleus to the cytoplasm of *Saccharomyces cerevisiae*. One of these genes, *MTR2*, has been cloned and shown to encode a novel 21-kDa nuclear protein that is essential for vegetative growth. MTR2 shows limited homology to a protein implicated in plasmid DNA transfer in *Escherichia coli*. PolyA$^+$RNA accumulates within the nucleus of *mtr2-1* in two to three foci at 37°C. mRNA, tRNA, and rRNA synthesis continue as do pre-mRNA splicing, tRNA processing, and rRNA export at 37°C. Under these conditions the polyA tail length increases, and protein synthesis is progressively inhibited. Nucleolar antigens also redistribute to two to three nuclear foci at 37°C, and this redistribution depends on ongoing transcription by RNA polymerase II. Surprisingly, these foci coincide with the sites of polyA$^+$RNA accumulation. Comparable colocalization and dependance on RNA polymerase II transcription is seen for the *mtr1-1* mutant. The disorganization of the nucleolus thus depends on mRNA accumulation in these mutants. We discuss the possible functions of MTR2 and the yeast nucleolus in mRNA export.

INTRODUCTION

The formation of functional mRNA in eukaryotes entails transcription, 5′- and 3′-end processing, splicing, nucleotide modifications, and transport to the cytoplasm. Very little is known about the mechanisms of mRNA transport, but we anticipate that this transport pathway depends on a succession of protein-RNA interactions that extend from the nucleosome to the external face of the nuclear envelope. To dissect this pathway, we and others initiated a genetic approach to identify conditional mRNA transport defective mutants (*rat* and *mtr* mutants) of *S. cerevisiae* (Amberg *et al.*, 1992; Kadowaki *et al.*, 1992, 1994). One such recessive mutant, *mtr1/prp20/srm1*, is characterized at 37°C by accumulation of polyA$^+$RNA in the nucleus and by multiple defects in RNA processing, reminiscent of the *rna1-1* mutant (Hopper *et al.*, 1990). The *MTR1* gene and its homologues appear to act as guanine nucleotide release proteins on nuclear GTPases of the *RAS* superfamily (Tartakoff and Schneiter, 1995). The *rat1* mutant also exhibits nuclear polyA$^+$RNA accumulation at the restrictive temperature (Amberg *et al.*, 1992), and, surprisingly, RAT1/HKE1 has exoribonuclease activity (Kenna *et al.*, 1993).

In the present report, we characterize a second recessive mutant, *mtr2-1*, and the *MTR2* gene. The consequences of mutation of *MTR2* with regard to RNA processing appear to be much more limited than for *MTR1*. Previous studies of the nucleolus have documented its fragmentation and "segregation" upon inhibition of RNA polymerase I and progression through mitosis (Busch and Smetana, 1970; Bell *et al.*, 1992; Oakes *et al.*, 1993).

MATERIALS AND METHODS

Fluorescent In Situ Hybridization and Dot Blot Analysis

Fluorescent in situ hybridization and dot blot analysis of polyA$^+$RNA were carried out as described (Kadowaki *et al.*, 1992).

RNase Protection Assay and Primer Extension

The *Xho* I-*Bgl* II DNA fragment of the yeast actin gene (Ng and Abelson, 1980) was cloned in pBluescript SKII$^+$ (pTACT1) and linearized

* Present address: Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115.

with *Xho* I. The ^{32}P-labeled probe was prepared using linearized plasmid and T3 RNA polymerase. Ten micrograms of total RNA, isolated from YPH 499, YTK200, and YTK108 and incubated 0, 0.5, or 2 h at 37°C, was used for hybridization and digested with RNase T1. The products were analyzed on a 5% polyacrylamide-8 M urea gel. Pre- and mature actin mRNA correspond to the 487 and 241 nucleotide bands, respectively.

Ten micrograms of total RNA isolated from Acc°, which contains a synthetic intron in the *lacZ* open reading frame (ORF), and pLGSD5-transformed SL7 and YTK203 were used for primer extension. The induction of *lacZ* transcription by 2% galactose for 0, 2, or 4 h was initiated after 20 min preincubation at 37°C. The methods and interpretation of extension products are based on Legrain and Rosbash (1989).

Analysis of rRNA and tRNA Processing

YPH499 and YTK200 were grown in SC-MET medium at 23°C, and 5×10^7 cells were preincubated at 2×10^7 cells/ml at 23 and 37°C for 20 min by adding equal volumes of 23 and 50°C prewarmed medium, respectively. ^{3}H-[methyl]methionine (0.1 mCi/ml) (72.2 Ci/mmol, New England Nuclear, Boston, MA) was then added. After 3 min 1 ml of cells was removed and chilled on ice. Cold methionine (0.5 mg/ml) was immediately added to the remaining volume and further 1-ml samples of cells were collected after 5 and 10 min. The labeled RNA was extracted, and then equal amounts of radioactivity were loaded in each lane on 1% Agarose gel.

Analysis of PolyA$^+$RNA

For tail length determination, 1 μg of total RNA isolated from YPH499 and YTK200 was labeled with ^{32}pCp (>1000 Ci/mmol, Amersham, Arlington Heights, IL) and RNA ligase, followed by RNase A/T1 digestion, as described (Minvielle-Sebastia *et al.*, 1991). The digestion products were ethanol precipitated with sonicated salmon sperm DNA as carrier and analyzed on a 12% polyacrylamide-8 M urea gel (Sachs and Davis, 1989).

Indirect Immunofluorescence

Fixation, spheroplasting, and attachment of cells to poly-L-lysine–coated slides were as for fluorescent in situ hybridization except cells were fixed for 2 h on ice for detection of HA-epitope–tagged MTR2. The slides were treated with methanol followed by 0.1% TX-100 in phosphate-buffered saline (PBS). Blocking was with 2% bovine serum albumin (BSA) in PBS. The monoclonal antibody YN9C5 (from J. Broach, Princeton Univ), recognizing an unidentified yeast nucleolar protein, A66 (recognizing NOP1), and 12CA5 (recognizing the HA-epitope) were diluted 200-fold (in PBS containing 2% BSA). A 100-fold dilution of fluorescein isothiocyanate (FITC)-conjugated anti mouse IgG (Boehringer Mannheim, Indianapolis, IN) was used for detection.

Detection of PolyA$^+$RNA and Nuclear Proteins

For combined detection of polyA$^+$RNA and nuclear proteins, cells were first processed as for fluorescent in situ hybridization. Primary antibodies were then added to a solution containing 1× SSC, 1% BSA, and 2 μg/ml FITC-avidin as for indirect immunofluorescence and incubated for 1 h at room temperature. Samples were then washed five times for 10 min with 1× SSC and reincubated for 1 h at room temperature with Lissamine-rhodamine B–conjugated anti-mouse IgG (Jackson Laboratory, Bar Harbor, ME) in 1× SSC, 1% BSA. Samples were finally washed twice with 2× SSC for 10 min each, twice with 2× SSC, 0.1% TX-100 for 10 min each, and finally twice with 2× SSC for 10 min each.

Identification, Cloning, and Sequencing of MTR2 Gene

YTK200 was transformed with a YCp50 yeast genomic DNA library (Rose and Broach, 1991), and plasmids were rescued from several *URA*$^+$ *TS*$^+$ transformants. All plasmids carried a common DNA fragment, and the complementing DNA fragment was identified by subcloning. The 3-kilobase (kb) *Eco*RI-*Xba* I fragment was cloned into the integrating vector, pRS306 (*URA3*) (Sikorski and Hieter, 1989), to yield pTK200 and then linearized upstream of the *MTR2* gene with *Bgl* II. The linearized plasmid was used for transformation of YPH259 (*ura3*-52), and resulting *URA*$^+$ transformants were crossed with YTK201 (*mtr2-1 ura3-52*). All tetrads (20) were parental ditype, indicating that plasmid DNA was integrated at the *MTR2* locus. The sequence of the *MTR2 Bgl* II-*Xba* I DNA fragment (1.8 kb) was determined using a series of nested deletions created by *Exo* III/Mung bean nuclease.

Disruption of the MTR2 Gene

The 3-kb *Eco*RI-*Xba* I DNA fragment including *MTR2* was cloned in pBluescript IISK$^+$ to yield pTK201. The internal *Bam*HI-*Bam*HI fragment was then replaced with a *Bgl* II-*Bgl* II DNA fragment carrying the *LEU2* gene from PS118 (Silver *et al.*, 1988) to yield pTK202. pTK202 was cut in vector sequences with *Sac* I and *Xho* I and used for transformation of YPH501. The disruption of *MTR2* in *LEU*$^+$ transformants was checked by Southern hybridization probing *Bgl* II/*Xba* I-digested genomic DNA with the *MTR2 Bgl* II-*Xba* I DNA fragment.

Epitope Tagging of MTR2

Epitope tagging of *MTR2* was performed by polymerase chain reaction (PCR), using linearized pTK201 and the following primers: primer 1, 5′ GGAGATCTTATGAACACCAATAGTAATA 3′; primer 2, 5′ GGAGATCTACTAAGCGTAGTCTGGGACGTCGTATGGGTAAATTTTTAGCA GAGAATCCT 3′; and primer 3, 5′ GGAGATCTACTAAATTTTTAGCAGAGAA 3′. The combination of primers 1/2 and primers 1/3 results in the production of C-terminal HA-epitope–tagged and untagged *MTR2*, respectively. PCR products were digested with *Bgl* II followed by cloning in pGAP316 (Kadowaki *et al.*, 1993). pTK203 (untagged *MTR2*) and pTK204 (tagged *MTR2*) were constructed by replacing the *GAL1* promoter with the *MTR2* promoter (*Eco*RI-*Bam*HI fragment). The construction of YTK205, YTK206, and YTK209 involved disrupting one copy of *MTR2* in YPH501 and transforming with pTK203, pTK204, and pTK211 followed by sporulation. *URA*$^+$ *LEU*$^+$ spores were recovered.

Subcellular fractionation of yeast cells was performed as described (Mirzayan *et al.*, 1992).

Yeast Strains

The original *mtr2-1* mutant was backcrossed three times with YPH strains to yield YTK200. Both temperature-sensitive growth and accumulation of polyA$^+$RNA in the nucleus at 37°C cosegregated 2:2 in these crosses. The double mutant (*mtr2-1 rpb1-1*) YTK202 was created by crossing YTK207 and RY260 (Nonet *et al.*, 1987) followed by sporulation. For construction of YTK203, the *mtr2-1* mutant allele was first rescued by introducing *Bgl* II- and *Bam*HI-gapped pTK205 (which carries a 3-kb *Eco*RI-*Xba* I fragment including all of *MTR2* in pRS316) into YTK200. The rescued DNA fragment containing the entire *mtr2-1* allele was then cloned in pBluescript SKII$^+$ to yield pTK206. pTK207 was then constructed by inserting a *Bgl* II-*Bgl* II fragment carrying *LEU2* from PS118 into the *Bgl* II site of pTK206. pTK207 was digested with *Sac* I and *Xho* I (which cut in vector sequences) and then used for transformation of SL7 to yield strain YTK203. Gene replacement was checked by Southern hybridization probing genomic DNA digested with *Eco*RI and *Xba* I with the *MTR2 Bgl* II-*Xba* I DNA fragment.

Plasmids, yeast strains, and relevant genotypes are shown in Tables 1 and 2.

RESULTS

Distribution and Characterization of PolyA$^+$RNA in the mtr2-1 Mutant

The *mtr2-1* mutant is one of the *mtr* mutants we isolated as described in our previous papers (Kadowaki *et al.*,

Table 1. List of plasmids used

pTACT1	*S. cerevisiae ACT1 Xho* I-*Bgl* II DNA fragment cloned in pBluescriptSKII$^+$
pTK200	*S. cerevisiae Eco*RI-*Xba* I DNA fragment including *MTR2* cloned in pRS306
pTK201	The same DNA fragment as pTK200 cloned in pBluescript SKII$^+$
pTK202	*Bam*HI-*Bam*HI DNA fragment of *MTR2* in pTK201 replaced by *LEU2 Bgl* II-*Bgl* II DNA fragment
pTK203	Wild-type *MTR2* ORF made by PCR, expressed from its own promoter, and terminated by *PGK* terminator, based on pRS316
pTK204	Same as pTK203, except *MTR2* is tagged with a HA epitope at its C-terminus
pTK205	The same DNA fragment as pTK200 cloned in pRS316
pTK206	The *mtr2-1* allele *Eco*RI-*Xba* I fragment cloned in pBluescriptSII$^+$
pTK207	*LEU2 Bgl* II-*Bgl* II DNA fragment cloned in the *Bgl* II site of pTK206
pTK209	Same as pTK203 except *MTR2* is under control of the *GAL1* promoter
pTK210	Same as pTK204 except *MTR2* is under control of the *GAL1* promoter
pTK211	Same as pTK204 except plasmid is based on

1992, 1994). At the restrictive temperature this mutant exhibits the most rapid accumulation of nuclear polyA$^+$RNA of all *mtr* mutants; accumulation of polyA$^+$RNA in the nucleus is evident after 20 min, and this signal remains unchanged for up to 3 h at 37°C. More than 90% of the cells exhibit nuclear polyA$^+$RNA accumulation. PolyA$^+$RNA characteristically accumulates as two or three foci in the nuclei of most cells (arrowheads in Figure 1). Such a signal is not visible when hybridization is with a biotinylated oligo-dA probe. To learn whether this nuclear polyA$^+$RNA signal is dependent on active RNA polymerase II, we have used the *rpb1-1* mutant, which is ts for RNA polymerase II (Nonet *et al.*, 1987); a *mtr2-1 rpb1-1* double mutant does not show strong nuclear accumulation of polyA$^+$RNA at the restrictive temperature, by comparison to parent *mtr2-1* (unpublished data). Furthermore, once polyA$^+$RNA has been accumulated in the *mtr2-1* nucleus by incubation at 37°C, the in situ hybridization signal persists upon continued incubation for 3 h at 37°C with the RNA polymerase inhibitor, thiolutin (Tipper, 1973) (unpublished data). To explore directly the possibility that the *mtr2-1* mutation enhances cytoplasmic mRNA degradation, we measured polyA$^+$-RNA content in wild-type (wt), *mtr2-1*, *rpb1-1*, and *mtr2-1 rpb1-1* strains by dot blot analysis. Analysis of the double mutant allows one to estimate the turnover rate of preexisting (i.e., predominantly cytoplasmic) polyA$^+$RNA. As shown in Figure 2, the total polyA$^+$RNA level remains roughly constant in *mtr2-1* during 3 h at 37°C, and the degradation rate of polyA$^+$RNA in *mtr2-1 rpb1-1* is comparable to that of *rpb1-1*.

An increase of average polyA tail length is also seen in *mtr2-1* within 30 min at 37°C (unpublished data). Protein synthesis of *mtr2-1* gradually stops at 37°C, as expected if the pool of cytoplasmic mRNA is progressively reduced (Kumagai, Kadowaki, and Tartakoff, unpublished results).

Table 2. Yeast strains and genotypes used

Yeast strains	Relevant genotype	Source
YPH259	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1*	P. Hieter
YPH499	*MATa ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63*	P. Hieter
YPH501	*MATa/α* $\frac{\textit{ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63}}{\textit{ura3-52 lys2-801 ade2-101 his-3Δ200 leu2-Δ1 trp1-Δ63}}$	"
YTK102	*MATα mtr1-1 ura3-52*	Kadowaki *et al.*, 1992
YTK105	*MATα mtr1-1 rpb1-1 ura3-52 lys2-801 ade2-101 his4-539*	Kadowaki *et al.*, 1992
YTK200	*MATa mtr2-1 ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ1*	This study
YTK201	*MATa mtr2-1 ura3-52 lys2-801 ade2-101 trp1-Δ1*	"
YTK202	*MATa mtr2-1 rpb1-1 ura3-52*	"
YTK203	*MATa ura3-52 leu2 mtr2-1::LEU2 GAL+*	"
YTK205	*MATα ura3-52 lys2-801 ade2-101 his3-Δ 200 leu2-Δ1 trp1-Δ63 ΔMTR2::LEU2* [pTK203]	"
YTK206	*MATα ura3-52 lys2-801 ade2-101 his3-Δ 200 leu2-Δ1 trp1-Δ63 ΔMTR2::LEU2* [pTK204]	"
YTK207	*MATα mtr2-1 ura3-52 lys2-801 ade2-101 his3-Δ 200 leu2-Δ1 trp1-Δ1*	"
YTK208	*MATa/α ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63 ΔMTR2::LEU2* / *ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63 MTR2*	This study
YTK209	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63 ΔMTR2::LEU2* [pTK211]	This study
SL7	*MATa ura3-52 leu2 GAL+*	S. Lemmon
RY260	*MATa rpb1-1 ura3-52*	R. Young
108	*MATα ade1 ade2 ura1 his7 lys2 tyr1 gal1 prp5-1*	*Yeast Genetic Stock Center*
EE1b (rnal-1)	*MATa rna1-1 rnh1::URA3 ura3-52 tyr1 his4 his7 ade1*	A. Hopper

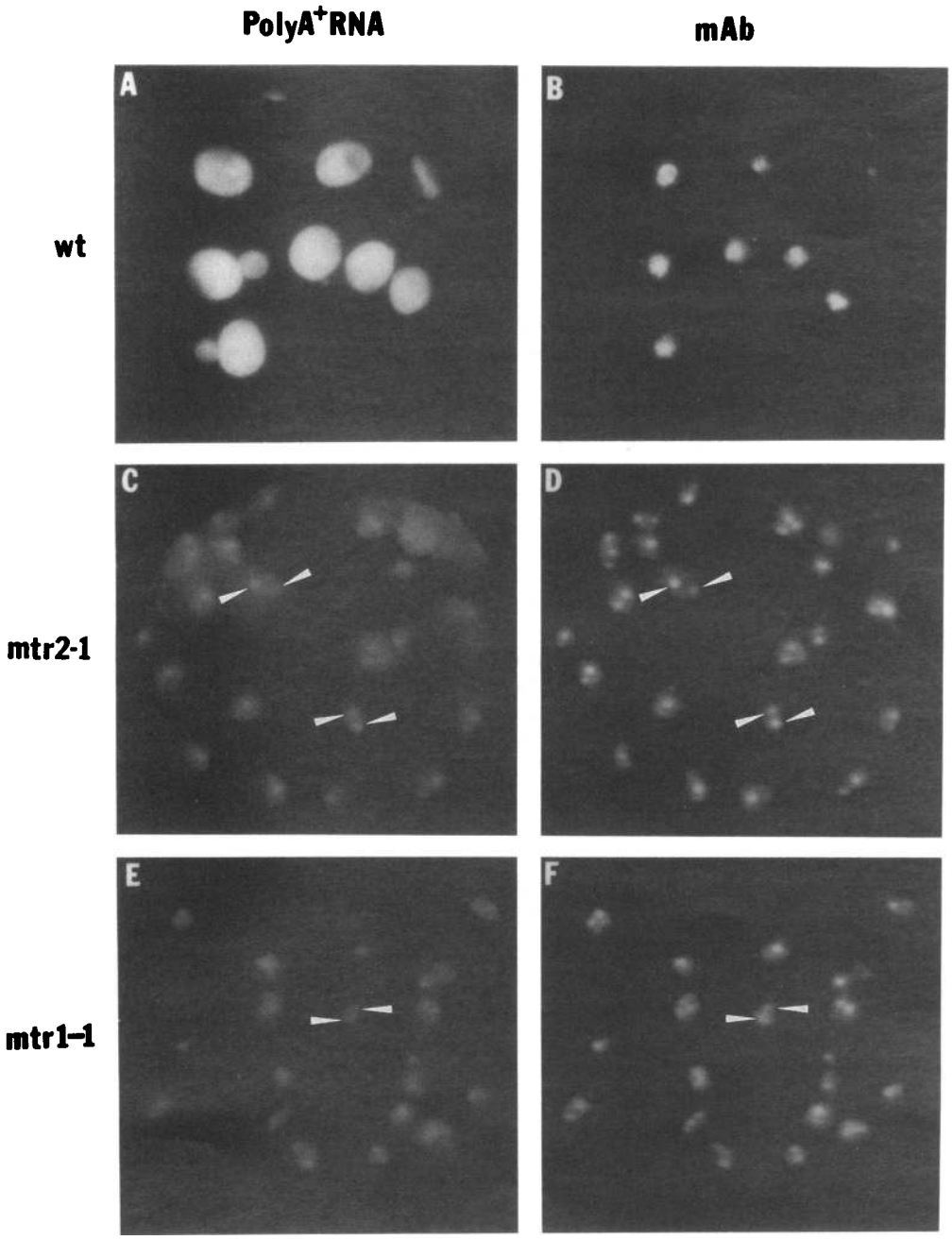

Figure 1. Colocalization of polyA$^+$RNA and nucleolar proteins. PolyA$^+$RNA and nucleolar antigens (detected with antibody YN9C5) were simultaneously detected in YPH499 (wt, A and B), YTK102 (*mtr2-1*, C and D), and YTK200 (*mtr1-1*, E and F) after 30 (YPH499 and YTK200) and 90 min (YTK102) incubation at 37°C. Comparable observations of colocalization with polyA$^+$RNA (arrowheads) have been made with a monoclonal antibody to NOP1.

Pre-mRNA Splicing in mtr2-1

To learn whether the *mtr2-1* mutation affects pre-mRNA splicing at 37°C, actin mRNA was analyzed by an RNase protection assay. As shown in Figure 3A, accumulation of actin pre-mRNA is not detected in *mtr2-1* after 0.5 and 2 h at 37°C in contrast to *prp5-1*, which is ts for splicing (Ruby *et al.*, 1993). Splicing of a newly-transcribed pre-mRNA was also checked by transforming wt cells and *mtr2-1* with a plasmid (Acc°) that carries a galactose-inducible *lacZ* gene containing a synthetic intron (Legrain and Rosbash, 1989). As shown in the primer extension experiment illustrated in Figure 3B, after incubation for 2 and 4 h at 37°C with galactose, equal amounts of mature *lacZ* mRNA (and pre-mRNA) are detected in wt and *mtr2-1*.

Because, upon splicing, the *lacZ* mRNA transcribed from Acc° codes for active β-galactosidase, we measured activity in Acc°-transformed cells and in cells transformed with its parent plasmid that lacks an intron, pLGSD5 (Guarente *et al.*, 1982). *mtr2-1* transformants

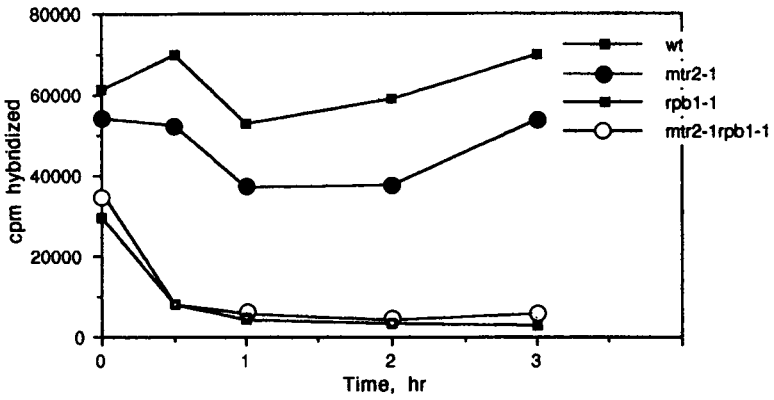

Figure 2. PolyA$^+$RNA in wt, *mtr2-1*, *rpb1-1*, and *mtr2-1 rpb1-1*. Dot blot analysis of polyA$^+$RNA content. Ten micrograms of total RNA isolated from YPH499 (wt), YTK200 (*mtr2-1*), RY260 (*rpb1-1*), and YTK202 (*mtr2-1 rpb1-1*) after incubation at 37°C for 0, 0.5, 1.0, 2.0, and 3.0 h were analyzed by dot blot using ^{32}P-labeled oligo-dT as a probe. The radioactivity of each sample was measured by scintillation counter and plotted for quantitative analysis. Note that total polyA$^+$RNA levels remain roughly unchanged during 3 h at 37°C in *mtr2-1* and that the decay rate in *rpb1* and *mtr2-1 rpb1-1* are similar. A pair of experiments gave essentially identical results.

incubated with galactose show very little activity at 37 compared to 23°C. This ratio is ~40 times higher in wild-type cells. This is consistent with the expectation that *lacZ* mRNA synthesized in the *mtr2-1* mutant is not exported.

tRNA and rRNA Processing in mtr2-1

rRNA processing was analyzed in ^{3}H-[methyl]methionine pulse-chase experiments after preincubation at 37°C. Synthesis of rRNA continues, but accumulation of ^{3}H-35S pre-rRNA and reduced production of ^{3}H-27, 25, 20, and 18S rRNA is evident in *mtr2-1* after 3 min pulse labeling. After 10 min chase, mature ^{3}H-25S and 18S rRNA are visible, but their appearance is slow compared to wt cells (Figure 4). Similar defects on rRNA processing are observed with ^{3}H-uridine–labeled RNA, indicating that the illustrated inhibition is not due to alteration of rRNA methylation. These changes may result from fragmentation of the nucleolus. Because processing of 20S–18S rRNA takes place in the cytoplasm (Udem and Warner, 1973) and 18S rRNA is produced at 37°C in this mutant, the *mtr2-1* mutation certainly does not completely block rRNA transport. Comparable experiments show that synthesis and processing of tRNA are not inhibited in *mtr2-1* (unpublished data).

Nucleolar Structure in mtr2-1

The yeast nucleolus forms an argentophilic crescent in contact with the nuclear envelope. It is thought to accomplish rRNA processing and ribosome assembly (Smitt *et al.*, 1973; Warner, 1989; Woolford, 1991). Because we and others have observed that nucleolar structure is severely disorganized in the *mtr1/prp20/srm1* mutant at 37°C (Oakes *et al.*, 1993; Kadowaki and Tartakoff, unpublished results), we have studied the organization of the nucleolus in *mtr2-1* using a monoclonal antibody that recognizes NOP1 (Aris and Blobel, 1988) and a monoclonal antibody that recognizes a pair of yeast nucleolar proteins (Broach, personal communication). The typical distribution of these antigens is unchanged in wt cells at 37°C, but it is grossly altered in *mtr2-1* (and *mtr1-1*) at 37°C (Figure 1). In most cells the nucleolus fragments to yield two or three foci. Comparable morphologic changes are not seen upon incubation with cycloheximide (Oakes *et al.*, 1993), upon inhibition of mRNA synthesis in *rpb1* at 37°C, or in wt treated with thiolutin. Corresponding morphological changes in the nucleolus of *mtr2-1* were detected by transmission electron microscopy and immunogold labeling (unpublished data).

Unexpectedly, after incubation of *mtr2-1* at 37°C, the accumulated polyA$^+$RNA actually colocalizes with the nucleolar fragments, whereas the chromatin-rich region of the nucleoplasm (4,6-diamidino-2-phenylindole [DAPI]-stained region) has much less polyA$^+$RNA (Figures 1 and 5). The same observation is also made in *mtr1-1* incubated at 37°C for 1.5 h (Figures 1 and 5). Thus, the sites of polyA$^+$RNA accumulation in *mtr2-1* and *mtr1-1* at 37°C appear to be concentrated in the nucleolus and distinct from the chromatin-rich nuclear region where most RNA polymerase II is located (Elliott *et al.*, 1992).

By performing parallel immunofluorescent studies of *mtr2-1 rpb1-1* and *mtr1-1 rpb1-1* double mutants, we observe that the nucleolar fragmentation occurs only when RNA polymerase II is active (Figure 5). Thus, loss of function of the MTR proteins does not itself alter the nucleolus. The nucleolar changes are secondary to strong nuclear accumulation of polyA$^+$RNA that is absent in *mtr2-1rpb1-1* (Figure 1) and *mtr1-1rpb1-1* (Kadowaki *et al.*, 1992).

Identification of the MTR2 Gene

To clone the *MTR2* gene, YTK200 (*mtr2-1*) was transformed with a YCp50 yeast genomic DNA library. A complementing 3.0-kb *Eco*RI-*Xba* I DNA fragment was recovered (Figure 6). Integration of this fragment into YPH259 followed by a genetic cross with YTK201 and analysis of tetrads proves that the complementing DNA encodes the *MTR2* gene (see MATERIALS AND METHODS). The sequence of the 1.8-kb *Bgl*II-*Xba*I

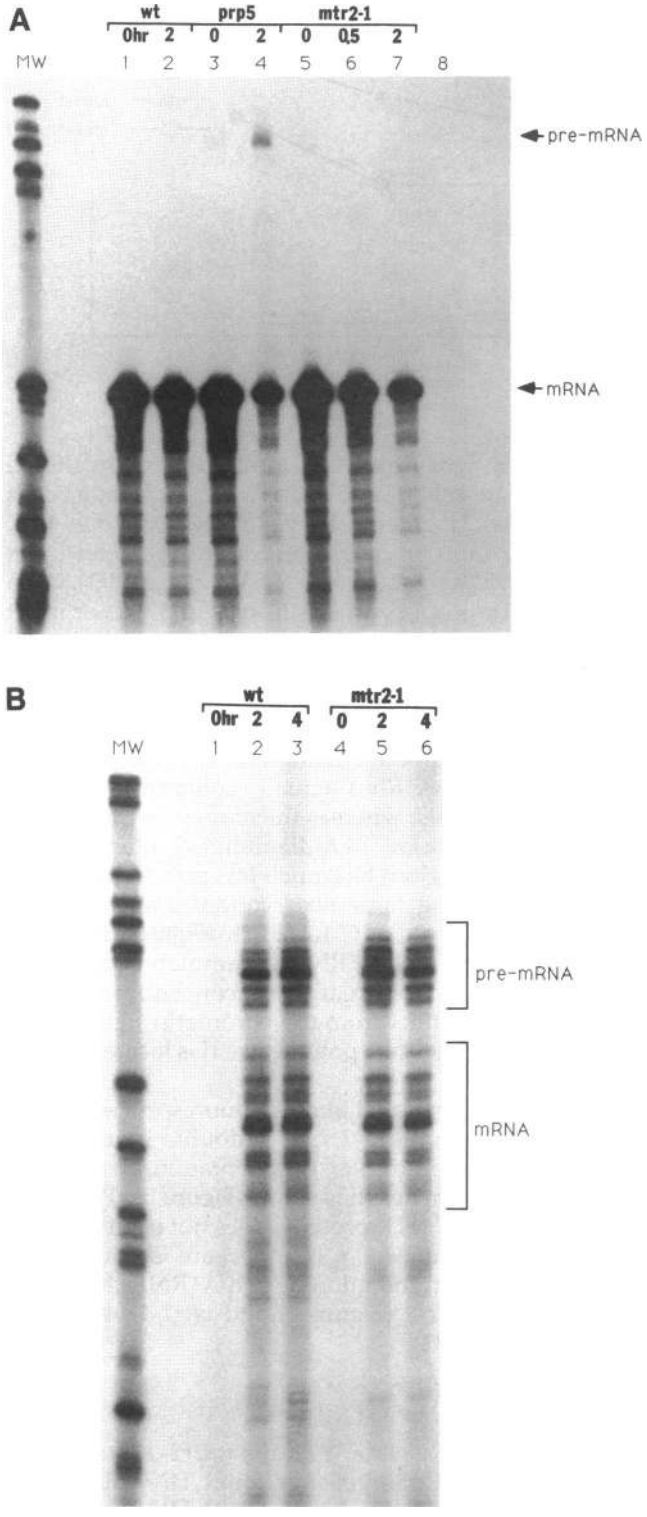

Figure 3. Pre-mRNA splicing in *mtr2-1*. (A) Detection of actin pre-mRNA and mature mRNA by RNase protection assay. Total RNA isolated from YPH499 (wt, lane 1 and 2), 108 (*prp5-1*, lane 3 and 4), and YTK200 (*mtr2-1*, lane 5 and 7) at 23°C and after 2 h incubation at 37°C was analyzed to detect actin pre-mRNA and mature mRNA. Lane 6 is total RNA isolated from YTK200 after 30 min incubation at 37°C. Yeast tRNA is used as a hybridization control (lane 8). MW designates ^{32}P-labeled *Hae* III fragments of pBR322. (B) Detection of *lacZ* pre-mRNA and mature mRNA by primer extension. Total RNA isolated from yeast transformed with a plasmid encoding a *lacZ* pre-mRNA with a synthetic intron (Acc°). SL7 (wt, lanes 1–3) and YTK203 (*mtr2-1*, lanes 4–6) 0, 2, and 4 h after galactose induction at 37°C were analyzed by primer extension. MW indicates ^{32}P-labeled *Hae* III fragments of pBR322. Both A and B document the continued production of spliced mRNA in *mtr2-1* at 37°C.

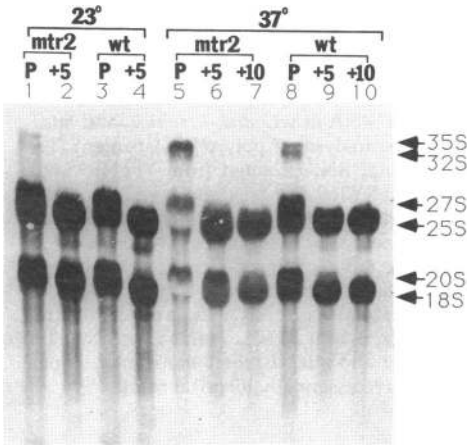

Figure 4. rRNA processing in *mtr2-1*. YTK200 (*mtr2-1*) and YPH499 (wt) were pulse-labeled with ^{3}H-methionine for 3 min (lanes 1 and 3, P) followed by 5 min chase (lanes 2 and 4, +5) at 23°C. YTK200 and YPH499 were also pulse-labeled for 3 min (lanes 5 and 8, P) after 20 min preincubation at 37°C followed by 5 (lanes 6 and 9, +5) and 10 min (lanes 7 and 10, +10) chase at 37°C. The labeled RNA (equal amounts of radioactivity) was analyzed on a 1% agarose gel with formaldehyde. Slowed processing is seen in the mutant, by comparison to wt.

DNA reveals one ORF that encodes a protein of 184 amino acids with a predicted molecular weight of 21 kDa (Figure 6). When this ORF is expressed from a *GAL1* promoter in *mtr2-1*, the transformant grows in galactose medium at 37°C but not on glucose. MTR2 is a novel largely hydrophilic protein without significant homology to known proteins by BLAST (Altschul *et al.*, 1990) and FASTA (Pearson and Lipman, 1988) search, except *E. coli* mbeA (Boyd *et al.*, 1989), which is necessary for plasmid transfer during bacterial conjugation. Two-thirds of MTR2 (120 amino acids) share 19.2% identity with N-terminal of mbeA (Figure 6). It has a putative nuclear localization signal (residues 136–139) but no obvious RNA-binding motif. The *MTR2* gene was physically mapped to the left arm of chromosome XI (Riles *et al.*, 1993). The codon adaptation index value of *MTR2* is 0.113 (Sharp and Li, 1987).

When one *MTR2* gene is disrupted (by insertion of *LEU2*) in a diploid strain and the diploid is then sporulated, only two spores are recovered from each tetrad. All viable spores are *LEU*$^{-}$, indicating that the *MTR2*

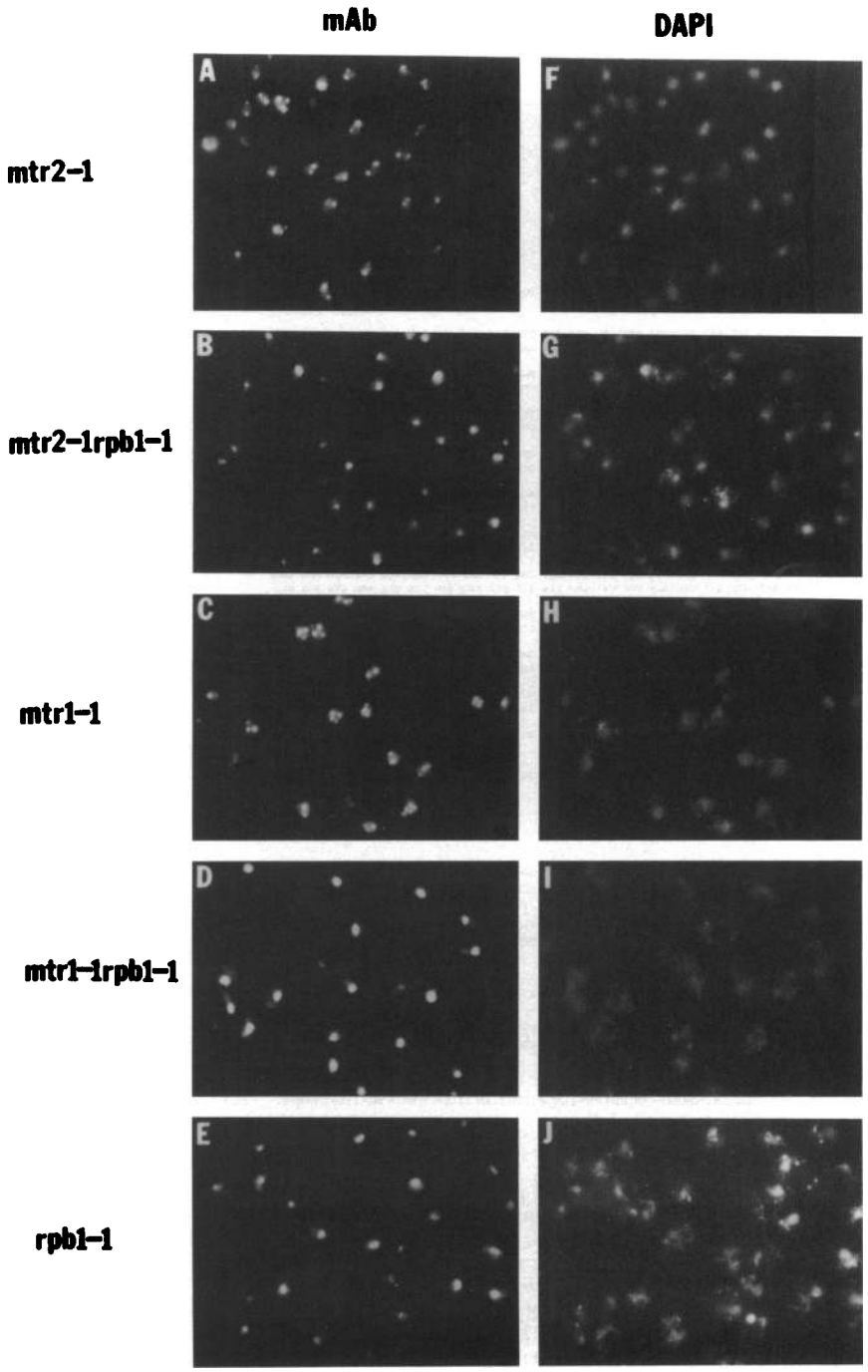

Figure 5. Distribution of nucleolar antigens in *mtr2-1* and *mtr2-1 rpb1-1*. The distribution of nucleolar antigens in YTK200 (*mtr2-1*, A and F), YTK202 (*mtr2-1 rpb1-1*, B and G), YTK102 (*mtr1-1*, C and H), YTK105 (*mtr1-1 rpb1-1*, D and I), and RY260 (*rpb1-1*, E and J) was monitored with monoclonal antibody YN9C5 (A–E). (Comparable observations were made with an anti-NOP1 antibody). Cells were incubated at 37°C for 30 (YTK200, YTK202) or 90 min (YTK102, YTK105, RY260). F–J are the DAPI images corresponding to A–E. Note that nucleolar fragmentation is not seen in the double mutant. It is also absent in *mtr1-1 rpb1-1* double mutants at 37°C.

gene is essential. In addition, when YTK205 and YTK206 (which have a disrupted chromosomal *MTR2* gene and carry a *URA3 MTR2* plasmid) were streaked on 5-fluoracetic acid (5-FOA) plates, no 5-FOA resistant colonies appeared (unpublished data). Thus, MTR2 is required for vegetative growth.

Localization of MTR2

To determine the intracellular localization of MTR2, a tagged *MTR2* gene bearing the HA epitope at its C-terminus was constructed. A centromeric plasmid carrying this construct can restore the viability of spores in which the endogenous *MTR2* gene is disrupted. At this low level of expression, detection of the HA-epitope–tagged MTR2 protein in YTK206 was not possible. After overexpression from a 2-μm plasmid, however, as shown in Figure 7A, MTR2 is detected by Western blotting in the crude nuclear fraction, just as is the nucleolar antigen, NOP1. To detect epitope-tagged MTR2

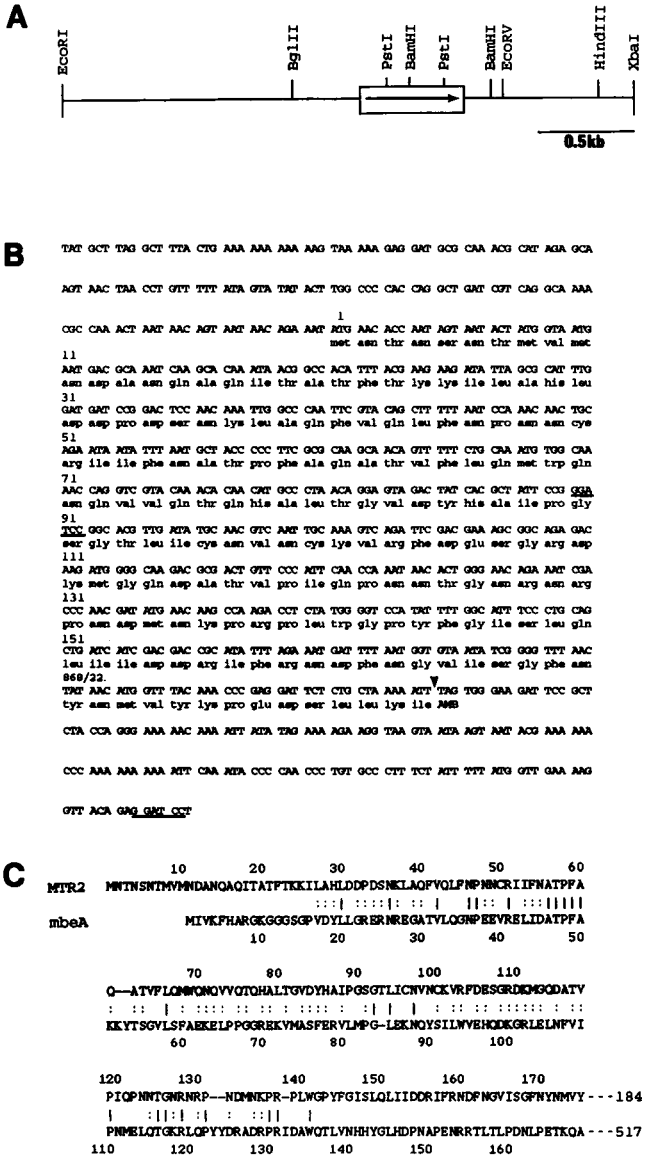

Figure 6. Sequence of the MTR2 gene. (A) Restriction map of the 3-kb *Eco*RI-*Xba* I DNA fragment containing the *MTR2* gene. The ORF of *MTR2* is shown by the open box, and the transcriptional direction is indicated by the arrow. (B) The nucleotide and predicted amino acid sequence of *MTR2*. The two *Bam*HI sites used for gene disruption are underlined. The insertion site of HA-epitope is shown by arrowhead. (C) Homology between MTR2 and *E. coli* mbeA. The sequence alignment was made by FASTA. The identical and similar amino acids between two proteins are shown by solid and dot line, respectively.

by immunofluorescence, it was necessary to drive its expression from a strong *GAL1* promoter. Figure 7B shows that the antigen is concentrated in the nucleus. Thus, although the overexpression of this protein might affect its intracellular localization, MTR2 is likely to be concentrated in the nucleus.

DISCUSSION

Inhibition of mRNA Export

The *mtr2-1* mutant was recovered by use of ^{3}H-amino acid suicide enrichment and screening by fluorescent in situ hybridization to detect nuclear accumulation of polyA$^+$RNA at 37°C (Kadowaki *et al.*, 1992, 1994). This nuclear polyA$^+$RNA signal suggests that *mtr2-1* is defective in mRNA export. Further evidence of the mRNA export block comes from the following: 1) the observation of inhibition of protein synthesis, 2) the lack of β-galactosidase activity in Acc° and pLGSD5 transformants at 37°C, despite ongoing *lacZ* mRNA synthesis, 3) subcellular fractionation experiments allowing evaluation of the distribution of ^{3}H-uridine–labeled polyA$^+$RNA, and 4) the polyA tail length increase.

The *mtr2-1* mutation does not affect pre-mRNA splicing nor tRNA processing at 37°C. In addition, the synthesis of mRNA, tRNA, and rRNA continue at the restrictive temperature. On the other hand, rRNA processing is somewhat slowed. This could be an indirect effect of nucleolar fragmentation or of reduced mRNA export, considering that the synthesis and processing of rRNA is under many controls (Warner, 1989; Woolford, 1991); however, some *mtr* mutants do not exhibit any obvious rRNA processing defect at the restrictive temperature. Because 18S rRNA is synthesized at 37°C, rRNA transport to the cytoplasm must continue, despite the nucleolar fragmentation for ≥30 min after temperature shift (Udem and Warner, 1973).

Intranuclear Distribution of polyA$^+$RNA

In *mtr2-1* the focal accumulation of polyA$^+$RNA in the nucleus is detected by in situ hybridization within 20 min at the restrictive temperature, and its pattern does not change during 3 h at 37°C. This is by contrast to *mtr1-1*, for which polyA$^+$RNA initially is focal and ultimately appears to fill the nucleus. Because the focal accumulation of polyA$^+$RNA in these mutants does not completely overlap with the DAPI-stained region, we suggest that the focal accumulation corresponds to a normally-occurring intermediate along the path of transport to the nuclear periphery. This intermediate may be exaggerated or distorted in the mutant background.

When mRNA synthesis occurs in *mtr1-1* and *mtr2-1* at 37°C, the nucleolus fragments and the accumulated polyA$^+$RNA coincides with foci containing nucleolar antigens. There are several ways to explain these observations; however, the simplest is that transport of polyA$^+$RNA in the nucleus normally involves nucleolar components and that an overabundance of nuclear polyA$^+$RNA leads to their disorganization. Possibly, polyA$^+$RNA actually passes through the nucleolus before export. A possible function of the nucleolus in transport and/or splicing of RNA other than rRNA is

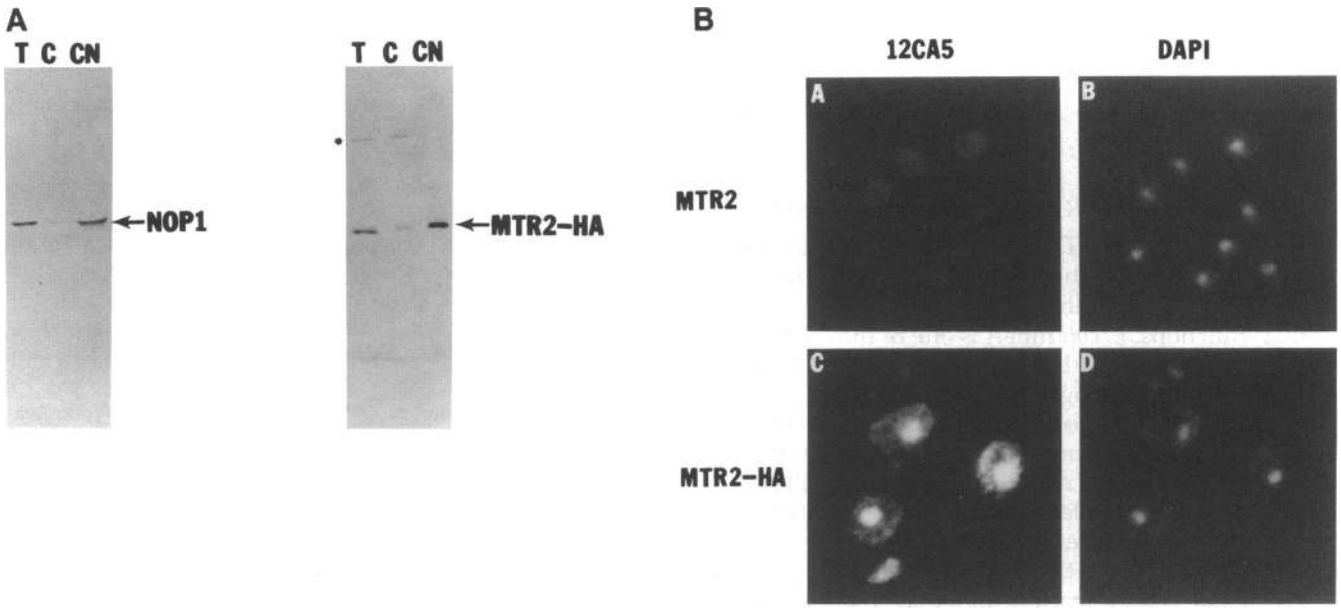

Figure 7. Localization of MTR2. (A) Subcellular localization of HA-epitope–tagged MTR2, subcellular fractionation. Fifty micrograms of protein of total (T), cytosol (C), and crude nuclear (CN) fractions obtained from YTK209 were separated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blot analysis using either A66 (anti-NOP1) or 12CA5 (anti-HA) monoclonal antibody. Both antigens are concentrated in the nuclear fraction. *, a cytosolic protein recognized by 12CA5 in addition to HA-epitope—tagged MTR2. (B) Subcellular localization of HA-epitope–tagged MTR2, indirect immunofluorescence. YTK203 (*mtr2-1*) transformed with either pTK209 (control, lacking epitope; A and B) or pTK210 (HA-tagged *MTR2*, C and D) was grown in 2% galactose medium at 30°C and then analyzed by indirect immunofluorescence using 12CA5. B and D illustrate the corresponding DAPI images. Note the nuclear concentration of tagged MTR2.

also suggested by several other observations: (1) in animal cells *myc* and *myoD* mRNAs are concentrated in the nucleolus (Bond and Wold, 1993), (2) nucleolar ablation interrupts gene expression (Deák *et al.*, 1972), (3) 5S and small nucleolar RNAs normally are concentrated in the nucleolus (Fournier and Maxwell, 1993), (4) in animal cells, the viral *rev* and *rex* proteins, which control splicing of viral pre-mRNA, include a nucleolar targeting sequence (Nosaka *et al.*, 1989), (5) a protein of the U1 snRNP has been reported to be concentrated in the nucleolus of yeast (Potashkin *et al.*, 1990), and (6) 2,2,7-trimethylguanosine–capped RNA (a determinant that is shared by snRNAs involved in mRNA processing and rRNA processing) (Fournier and Maxwell, 1993) is concentrated in the nucleolus of yeast (Potashkin *et al.*, 1990). In addition, our ongoing studies have shown that polyA$^+$RNA is normally detectable in the yeast nucleolus and that a conditional mutation in the nucleolar protein, MTR3, leads to accumulation of polyA$^+$RNA in association with nucleolar antigens.

If MTR2 is essential for transit of polyA$^+$RNA through the nucleolus, the buildup of polyA$^+$RNA in the *mtr2-1* nucleolus could force its fragmentation. Moreover, if MTR2 normally binds both polyA$^+$RNA and nucleolar components, it is possible that the nucleolar proteins that we detect require MTR2 for their coherence (i.e., in the presence of excessive amounts of polyA$^+$RNA in the mutant background, MTR2 function becomes limiting and the nucleolus fragments. Additionally, like certain nuclear and nucleolar proteins of animal cells (Borer *et al.*, 1989; Pinol-Roma and Dreyfuss, 1992; Schmidt-Zachman *et al.*, 1993), the nucleolar proteins studied here may normally cycle between the nucleus and cytoplasm. If their transport requires MTR2—most of which may be sequestered by the accumulated polyA$^+$RNA—the foci to which they redistribute may be an intermediate along the path of their return from the cytoplasm to the nucleolus.

Nevertheless, nucleolar organization and polyA$^+$RNA distribution/processing are not always closely linked. For example, an anatomically normal nucleolus is absent in RNA-polymerase I deficient strains that, nevertheless, are viable if rRNA is transcribed by RNA-polymerase II (Oakes *et al.*, 1993). Extensive depletion and temperature-sensitive mutations of the essential multifunctional nucleolar protein, NOP1 (Tollervey *et al.*, 1991, 1993), do not lead to nuclear accumulation of polyA$^+$RNA. Interruption of rRNA processing in ts mutants *rrp2* (Shuai and Warner, 1991) and *rrp1* (Fabian and Hopper, 1987) does not lead to nuclear accumulation of polyA$^+$RNA, and interruption of mRNA synthesis in *rpb1-1* (Nonet *et al.*, 1987) at 37°C or by use of thiolutin does not cause nucleolar fragmentation. As

mentioned in the INTRODUCTION, fragmentation of nucleoli is characteristically linked to inhibition of RNA polymerase I. The example of *mtr1* and *mtr2* demonstrates that other factors also control nucleolar coherence.

Identification of MTR2 and Its Possible Functions

MTR2 is a novel 21-kDa essential protein that is likely to be concentrated in the nucleus. The modest homology between MTR2 and mbeA suggests that they may share similar functions. *E. coli* mbeA is one of the mobilization (*mob*) gene products that are thought to be necessary for the specific nicking at *oriT*, directing the 5′ end of the nicked strand into the recipient, recircularization, and the priming of complementary strand synthesis in the recipient (Boyd *et al.*, 1989). One possibility is that MTR2 may act in a related way and pilot mRNA transport in the nucleus after transcription. Further work will be needed to address this hypothesis.

Nucleolar fragmentation and polyA$^+$RNA colocalization with nucleolar proteins are observed in both *mtr2-1* and *mtr1-1*, which has a defect in a putative guanine nucleotide release protein which acts on small nuclear GTPases (CNR1/2) (Kadowaki *et al.*, 1993). MTR2 could be a down-stream effector or a GAP (GTPase-activating protein) of these GTPases, but no suppression of temperature sensitivity was observed in *mtr2-1* transformed with high copy number plasmids carrying either *MTR1* or *CNR1/2*. Similarly, transformation of *mtr1-1* with a high copy number plasmid carrying *MTR2* did not rescue its temperature-sensitive growth.

ACKNOWLEDGMENTS

We thank P. Hieter for yeast strains and plasmids, S. Lemmon, A. Hopper, D. Tollervey, J. Warner, M. Nomura, and R. Young for yeast strains, M. Rose for the YCp50 library, J. Broach for the anti-nucleolar antibody YN9C5, J. Aris for the anti-NOP1 antibody and advice on electron microscopy, D. Templeton for the 12CA5 antibody, M. Grunstein for the anti-histone H2B antibody, U. Stochaj for the anti-NSP1 peptide antibody, M. Rosbash for the Acc° plasmid, P. Silver for the PS118 plasmid, N. Belcher for thiolutin, L. Riles for prime lambda-clone filters, S. Chen for technical help, G. Chanfreau for comments on the text, F. Rottman and R. Ludwiczak for help with the 5′ methyl cap analysis, M. Ward for preparing this manuscript, and the National Institutes of Health for grant GM-46569.

REFERENCES

Aebi, M., Clark, M.W., Vijayraghavan, U., and Abelson, J. (1990). A yeast mutant, *PRP20*, altered in mRNA metabolism and maintenance of the nuclear structure, defective in a gene homologous to human gene RCC1 which is involved in the control of chromosome condensation. Mol. Gen. Genet. *224*, 72–80.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. *215*, 403–410.

Amberg, D.C., Goldstein, A.L., and Cole, C.N. (1992). Isolation and characterization of *RAT1*: an essential gene of *Saccharomyces cerevisiae* required for the efficient nucleocytoplasmic trafficking of mRNA. Genes & Dev. *6*, 1173–1189.

Aris, J.P., and Blobel, G. (1988). Identification and characterization of a yeast nucleolar protein is similar to a rat liver nucleolar protein. J. Cell Biol. *107*, 17–31.

Bell, P., Dabauvalle, M.-C., and Scheer, U. (1992). In vitro assembly of prenucleolar bodies in *Xenopus* egg extract. J. Cell Biol. *118*, 1297–1304.

Bond, V.C., and Wold, B. (1993). Nucleolar localization of *myc* transcripts. Mol. Cell. Biol. *13*, 3221–3230.

Borer, R.A., Lehner, C.F., Eppenberger, H.M., and Nigg, E.A. (1989). Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell *56*, 379–390.

Boyd, A.C., Archer, J.A.K., and Sherratt, D.J. (1989). Characterization of the ColE1 mobilization region and its protein products. Mol. Gen. Genet. *217*, 488–498.

Busch, H., and Smetana, K. (1970). The Nucleolus, New York: Academic Press.

Deák, I., Sidebottom, E., and Harris, H. (1972). Further experiments on the role of the nucleolus in the expression of structural genes. J. Cell Sci. *11*, 379–391.

Elliott, D.J., Bowman, D.S., Abovich, N., Fay, F.S., and Rosbash, M. (1992). A yeast splicing factor is localized in discrete subnuclear domains. EMBO J. *11*, 3731–3736.

Fabian, G.R., and Hopper, A.K. (1987). *RRP1*, a *Saccharomyces cerevisiae* gene affecting rRNA processing and production of mature ribosomal subunits. J. Bacteriol. *169*, 1571–1578.

Fournier, M.J., and Maxwell, E.S. (1993). The nucleolar snRNAs: catching up with the splicesomal snRNAs. Trends Biol. Sci. *18*, 131–135.

Guarente, L., Yocum, R., and Gifford, P. (1982). A GAL10-CYC1 hybrid yeast promoter identifies the GAL4 regulatory region as an upstream site. Proc. Natl. Acad. Sci. USA *79*, 7410–7414.

Hopper, A.K., Traglia, H.M., and Dunst, R.W. (1990). The yeast *RNA1* gene product necessary for RNA processing is located in the cytosol and apparently excluded from the nucleus. J. Cell Biol. *111*, 309–321.

Kadowaki, T., Chen, S., Hitomi, M., Jacobs, E., Kumagai, C., Liang, S., Schneiter, R., Singleton, D., Wisniewska, J., and Tartakoff, A.M. (1994). Isolation and characterization of *Saccharomyces cerevisiae* mRNA transport-defective (*mtr*) mutants. J. Cell Biol. *126*, 649–659.

Kadowaki, T., Spitz, L., Goldfarb, D., Tartakoff, A.M., and Ohno, M. (1993). Regulation of RNA processing and transport by a nuclear guanine nucleotide release protein and Ras superfamily. EMBO J. *12*, 2929–2937.

Kadowaki, T., Zhao, Y., and Tartakoff, A. (1992). A conditional yeast mutant deficient in mRNA transport from nucleus to cytoplasm. Proc. Natl. Acad. Sci. USA *89*, 2312–2316.

Kenna, M., Stevens, A., McCammon, M., and Douglas, M.G. (1993). An essential yeast gene with homology to the exonuclease-encoding *XRN1/KEM1* gene also encodes a protein with exoribonuclease activity. Mol. Cell. Biol. *13*, 341–350.

Legrain, P., and Rosbash, M. (1989). Some *Cis*- and *Trans*-acting mutants for splicing target pre-mRNA to the cytoplasm. Cell *57*, 573–583.

Matsumoto, T., and Beach, D. (1991). Premature initiation of mitosis in yeast lacking RCC1 or an interacting GTPase. Cell *66*, 347–360.

Minvielle-Sebastia, L., Winsor, B., Bonneaud, N., and Lacroute, F. (1991). Mutations in the yeast *RNA14* and *RNA15* genes result in an abnormal decay rate; sequence analysis reveals an RNA-binding domain in the RNA15 protein. Mol. Cell. Biol. *11*, 3075–3087.

Mirzayan, C., Copeland, C.S., and Snyder, M. (1992). The *NUF1* gene encodes an essential coiled-coil related protein that is a potential component of the yeast nucleoskeleton. J. Cell Biol. *116*, 1319–1332.

Myers, A.M., Tzagoloff, A., Kinney, D.M., and Lusty, C.J. (1986). Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene *45*, 299–310.

Ng, R., and Abelson, J. (1980). Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *77*, 3912–3916.

Nonet, M., Scafe, C., Sexton, J., and Young, R. (1987). Eukaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol. Cell. Biol. *7*, 1602–1611.

Nosaka, T., Siomi, H., Adachi, Y., Ishibashi, M., Kubota, S., Maki, M., and Hatanaka, M. (1989). Nucleolar targeting signal of human T-cell leukemia virus type I *rex*-encoded protein is essential for cytoplasmic accumulation of unspliced viral mRNA. Proc. Natl. Acad. Sci. USA *86*, 9798–9802.

Oakes, M., Nogi, Y., Clark, M.W., and Nomura, M. (1993). Structural alterations of the nucleolus in mutants of *Saccharomyces cerevisiae* defective in RNA polymerase I. Mol. Cell. Biol. *13*, 2441–2455.

Pearson, W.R., and Lipman, D.J. (1988). Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA *85*, 2444–2448.

Pinol-Roma, S., and Dreyfuss, G. (1992). Shuttling of pre-mRNA binding proteins between the nucleus and cytoplasm. Nature *355*, 730–732.

Potashkin, J.A., Derby, R.J., and Spector, D.L. (1990). Differential distribution of factors involved in pre-mRNA processing in the yeast cell nucleus. Mol. Cell. Biol. *10*, 3524–3534.

Riles, L., Dutchik, J.E., Baktha, A., McCauley, B.K., Thayer, E.C., Leckie, M.P., Braden, V.V., Depke, J.E., and Olson, M.V. (1993). Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. Genetics *134*, 81–150.

Rose, M.D., and Broach, J.R. (1991). Cloning genes by complementation in yeast. Methods Enzymol. *194*, 195–230.

Ruby, S., Chang, T.-H., and Abelson, J. (1993). Four yeast splicesomal proteins interact to promote U2 snRNA binding to pre-mRNA. Genes & Dev. *7*, 1909–1925.

Sachs, A., and Davis, R. (1989). The polyA binding protein is required for poly(A) shortening and 60s ribosomal subunit dependent translation initiation. Cell *58*, 857–867.

Schmidt-Zachmann, M.S., Dargemont, C., Kühn, L.C., and Nigg, E.A. (1993). Nuclear export of proteins: the role of nuclear retention. Cell *74*, 493–504.

Sharp, P.M., and Li, W.-H. (1987). The codon adaptation index—a measure of directional synonymous codon usage bias, and its potential applications. Nucleic Acids Res. *15*, 1281–1295.

Shuai, K., and Warner, J.R. (1991). A temperature sensitive mutant of *Saccharomyces cerevisiae* defective in pre-rRNA processing. Nucleic Acids Res. *19*, 5059–5064.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Silver, P.A., Chiang, A., and Sadler, I. (1988). Mutations that alter both localization and production of a yeast nuclear protein. Genes & Dev. *2*, 707–717.

Smitt, W.W.S., Vlak, J.M., Molenaar, I., and Rozijn, T.H. (1973). Nucleolar function of the dense crescent in the yeast nucleus. Exp. Cell Res. *80*, 313–321.

Tartakoff, A., and Schneiter, R. (1995). The nuclear GTPase cycle. Trends Cell Biol. (*in press*).

Tipper, D. (1973). Inhibition of yeast RNA polymerases by thiolutin. J. Bacteriol. *116*, 245–256.

Tollervey, D., Lehtonen, H., Carmo-Fonseca, M., and Hurt, E.C. (1991). The small nucleolar RNP protein NOP1 (fibrillarin) is required for pre-rRNA processing in yeast. EMBO J. *10*, 573–583.

Tollervey, D., Lehtonen, H., Jansen, R., Kern, H., and Hurt, E.C. (1993). Temperature-sensitive mutations demonstrate roles for yeast fibrillarin in pre-rRNA processing, pre-rRNA methylation, and ribosome assembly. Cell *72*, 443–457.

Udem, S.A., and Warner, J.R. (1973). The cytoplasmic maturation of a ribosomal precursor ribonucleic acid in yeast. J. Biol. Chem. *248*, 1412–1416.

Warner, J. (1989). Synthesis of ribosomes in *Saccharomyces cerevisiae*. Microbiol. Rev. *53*, 256–271.

Woolford, J.L., Jr. (1991). The structure and biogenesis of yeast ribosomes. Adv. Genet. *29*, 63–118.