Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2312–2316, March 1992
Cell Biology

A conditional yeast mutant deficient in mRNA transport from nucleus to cytoplasm

(mRNA stability/*in situ* hybridization)

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Communicated by George E. Palade, November 18, 1991

ABSTRACT **Transport of mRNA from nucleus to cytoplasm is critical for eukaryotic gene expression; however, the mechanism of export is unknown. Selection and screening procedures have therefore been used to obtain a family of temperature-sensitive conditional mutants of *Saccharomyces cerevisiae* that accumulate poly(A)$^+$ RNA in the nucleus when incubated at 37°C, as judged by *in situ* hybridization. In one such *m*RNA *t*ransport mutant, *mtr1-1*, RNA synthesis continues, the export of poly(A)$^+$ RNA is inhibited, intranuclear poly(A)$^+$ is remarkably stable, and protein synthesis gradually stops. Thus, there is no tight coupling between RNA synthesis and export. The export lesion is reversible. Although mRNA export is clearly not a default option, neither inhibition of protein synthesis, inhibition of mRNA splicing, nor inhibition of poly(A)-binding protein function blocks export of the average poly(A)$^+$, as judged by *in situ* hybridization. Further analysis of the family of *mtr* mutants should help map the path of RNA transport.**

Gene expression in eukaryotes requires the rapid and selective export of mRNA from the nucleus to the cytoplasm. Most pre-mRNAs acquire a 5′ m^{7}G cap and are cleaved and polyadenylylated to generate their 3′ end. mRNAs have few other common structural features; however, specific proteins are associated with both extremities of intranuclear pre-mRNAs (1, 2), and intranuclear mRNAs associate with a number of additional proteins (3, 4) as well as the karyoskeleton (5).

Export of 5S RNA and rRNAs requires association with specific proteins (6–8) and the export of tRNA is highly sensitive to base changes, possibly for this reason (9). mRNA export is facilitated by its 5′ m^{7}G cap (10, 11). Exit of tRNAs and ribosomal subunits appears to be receptor mediated (9, 12).

Because colloidal gold coated with poly(A), tRNA, or 5S RNA microinjected into the nucleus exits via nuclear pores (13) and because exit of tRNA and 5S RNA is inhibited by antibodies that react with pore complexes (14), it is likely that mRNA also exits via pores. Maximal mRNA release from isolated animal cell nuclei requires ATP (15–17).

In *Saccharomyces cerevisiae* several features of processing of pre-mRNA are relatively simple: only the 5′ extremity of mRNA is methylated (18), only few mRNAs undergo splicing (19, 20), and the 3′ poly(A) tail of yeast mRNA is somewhat shorter than in animal cells (1, 21). Although nuclear pores of yeast have been characterized (22–24), and although temperature sensitive (ts) splicing mutants (*prp* mutants) have been produced (19, 20, 25–27), only a single ts mutant has been claimed to affect mRNA export (28). This mutant, *rna1*, has multiple defects in covalent processing of RNA. The corresponding gene product is cytoplasmic (29).

METHODS

Selection of *mtr* Mutants. The YPH1/2 and YPH258/9 strains (wild type; ref. 30) were treated with 3% ethyl methanesulfonate. Two selection procedures were then used. (*i*) Selection A. Cells were incubated 3 hr at 37°C in SD medium (31) and labeled 30 min at 37°C with [^{3}H]lysine in lysine-free SD medium. The cells were then washed and stored in 20% glycerol/YPAD medium (31) at −80°C. When survival was <1%, replica plates were prepared to screen for ts growth on SD medium. (*ii*) Selection B. Cells were incubated 3 hr at 37°C in lysine-free SD medium supplemented with the lysine analogue *S*-2-aminoethyl-L-cysteine and the proline analogue L-azetidine-2-carboxylate. The incubation was continued at 37°C for 24 hr; then cells were inoculated to YEPD plates. The survival fraction of the cells after this treatment was 0.1–0.2%. Replica plates were made to screen for ts growth on SD medium.

To evaluate protein synthesis, individual colonies were labeled in complete SD medium supplemented with ^{3}H-labeled amino acid mixture. Aliquots were spotted onto filter paper and processed by trichloroacetic acid precipitation.

Genetic Procedures. The original mutant, ts17a, was backcrossed twice with YPH47 and then once with BJ2664 to yield the YTK strain series (*mtr1-1*) (31). The double mutant YTK 105 (*rpb1-1 mtr1-1*) was constructed by a cross between the two ts parents. Tetrads were selected that included two ts and two Ts$^+$ spores. The ts spores were further crossed to YTK103, YTK104 (*mtr1-1*) and RY260, RY262 (*rpb1-1*; ref. 32) to verify their genotypes.

***In Situ* Hybridization and 4′,6-Diamidino-2-Phenylindole (DAPI) Staining.** Cells were fixed in suspension with formaldehyde, washed in 1.2 M sorbitol/0.1 M potassium phosphate, pH 7.5 (solution A), "spheroplasted," spotted onto poly(L-lysine)-coated glass slides, dehydrated through 100% ethanol, and dried. Ten microliters of hybridization solution consisting of 3′-biotinylated (dT)$_{25-30}$, yeast tRNA, salmon sperm DNA, 2× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), vanadyl complex, bovine serum albumin, and dextran sulfate was then added. Each preparation was then washed with 2× SSC, 1× SSC, and, briefly, 4× SSC/0.1% Triton X-100. Fluorescein isothiocyanate-avidin was added for 30 min at room temperature. The preparation was washed with 4× SSC, 4× SSC/0.1% Triton X-100, and 4× SSC, mounted in 90% glycerol, 1 mg of *p*-phenylenediamine per ml, 45 ng of DAPI per ml, and PBS, and examined.

Effect of Inhibition of ATP Production on Poly(A)$^+$ RNA Distribution. YTK101 was cultured in SD medium at 23°C. An equal volume of 55°C SD medium was added and incubated at 37°C for 2 hr. The cells were washed once with 37°C water and then washed with glucose-free SD medium con-

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Abbreviations: ts, temperature sensitive; wt, wild type; PABP, poly(A)-binding protein; DAPI, 4′,6-diamidino-2-phenylindole.

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taining cycloheximide and suspended in the same medium at 37°C. Part of the cell suspension was fixed. The rest received an equal volume of 4°C 4% glucose SD medium containing cycloheximide or 4°C glucose-free SD medium containing dinitrophenol, deoxyglucose, and cycloheximide. The incubation was continued at 23°C until the cells were fixed for *in situ* hybridization.

Reversibility of Protein Synthesis. YTK102 was grown overnight in SD medium at 23°C. An equal volume of 55°C SD medium was then added and the incubation was continued at 37°C for 1–3 hr. After the appropriate interval, an equal volume of 4°C SD medium containing 6 μg of thiolutin per ml and ^{3}H-labeled amino acid mixture was added. Culture at 23°C was continued for 0–6 hr followed by determination of acid-insoluble cpm.

Analysis of β-Galactosidase Expression. Strains YTK100 and YTK102 were transformed with pLGSD5 (33) and grown in uracil-free medium containing 2% raffinose to an OD 600 nm of ≈1 at 23°C. Galactose induction was achieved by adding an equal volume of 23°C or 55°C 4% galactose in uracil-free medium.

To evaluate the reversibility of *lacZ* mRNA transport at 23°C, galactose induction of cells transformed with pLGSD5 was performed for 2.5 hr at 37°C, as above. Control samples remained in raffinose-containing medium at 37°C. For the 1- to 7-hr reincubation interval at 23°C, uracil-free medium was then added. During this interval all samples contained 1.5% raffinose, 1% galactose, and 2% glucose. β-Galactosidase activity was measured and expressed as in ref. 31.

RNA Analysis. Total RNA was extracted (34), dot-blotted to nylon membrane, and UV cross-linked. Oligo(dT) end-labeled with [α-^{32}P]TTP was used for hybridization in 2× SSPE (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA), 5× Denhardt's solution, 1% SDS, and 0.1 mg of salmon sperm DNA per ml at 37°C. The membrane was then washed with 2× SSC/1% SDS at room temperature, 2× SSC/1% SDS at 37°C, and then 1× SSC/1% SDS at room temperature.

To evaluate synthesis of poly(A)$^+$ RNA, tRNA, and rRNA, wild type (wt) and *mtr1-1* were grown in low phosphate YEPD medium (35) at 23°C and then incubated 0–90 min at 23°C or 37°C in low phosphate YEPD medium prior to addition of $^{32}P_i$. After 2 min and 10 min samples were washed by filtration using 0.1% Na_2HPO_4/100 μg of cycloheximide per ml in 50 mM sodium acetate, pH 5.3/10 mM EDTA. Total RNA was extracted and analyzed on 1% formaldehyde/agarose and 10% acrylamide/7 M urea gels after elimination of polyphosphate (36) or fractionated on oligo(dT)-cellulose (32, 37).

RESULTS

Selection of mRNA Transport (*mtr*) Mutants. To enrich for mutants that cannot export mRNA at 37°C, we have used "suicide" selection procedures based on the supposition that cells that have not exported mRNA for 3 hr will tolerate incubation in the presence of high concentrations of ^{3}H-labeled amino acids or toxic amino acid analogues. In a typical experiment, ^{3}H-labeled amino acid suicide selection of 2.5×10^5 mutagenized cells gave 9 that exhibited progressive slowing of protein synthesis at 37°C and intranuclear accumulation of poly(A)$^+$ RNA at 37°C. A dozen complementation groups of recessive mutants have been obtained. The data presented below concern one of several alleles of one such ts mutant, ts17a, that was backcrossed to yield the YTK series of strains (*mtr1-1*). These strains exhibited 2:2 cosegregation of ts growth and accumulation of poly(A)$^+$ RNA in the nucleus after 3 hr at 37°C upon dissection of 20 tetrads from the last backcross.

Basic Phenotype of *mtr1-1*. Fig. 1*A* illustrates the kinetics of protein synthesis of *mtr1-1* by comparison to wt at 23°C and 37°C. Synthesis is quasilinear except for *mtr1-1*, which stops after 1–2 hr at 37°C. Comparable kinetics are seen for a mutant in which RNA polymerase II is ts (*rpb1-1*; RY260; ref. 32) and for *rna1* (not shown).

In situ hybridization to detect poly(A)$^+$ RNA revealed a uniform distribution of fluorescence in wt cells, *rpb1-1*, or *mtr1-1* grown at 23°C (Fig. 1*B*, panels 1 and 3). In *rpb1-1*, incubation at 37°C leads to a progressive disappearance of fluorescence (panel 2). Moreover, in all cases fluorescence was eliminated by pretreatment with nonspecific RNase (not shown). When *mtr1-1* cells were incubated for increasing periods at 37°C, the cytoplasmic signal faded and the nuclear signal increased for at least 3 hr [compare panel 3 (23°C) with panels 4 and 7 (3 hr, 37°C)]. During this period, the intranuclear signal grows from a focal spot (+40 min) to a more complex often multilobed structure (+80 min) (not shown), which ultimately fills the entire nucleoplasm (panel 6). The accentuated nuclear signal was not seen with a double mutant, *rpb1-1 mtr1-1* (panel 5; 3 hr, 37°C), or when the 37°C incubation of *mtr1-1* was in the presence of 3 μg of thiolutin per ml, an RNA polymerase inhibitor that has little effect on RNA turnover (37, 38). Since the production of the intranuclear signal requires active RNA polymerase II and is sensitive to RNase we consider that it is poly(A)$^+$ RNA.

To learn whether poly(A)$^+$ RNA export is interrupted at 37°C, *mtr1-1* was incubated 2 hr at 37°C to yield a strong intranuclear signal and then maintained at 37°C with 3 μg of thiolutin per ml for 1–3 hr. The intense nuclear signal persisted during this period (not shown). Thus, the intranuclear poly(A)$^+$ RNA is surprisingly stable in *mtr1-1* at 37°C. Such a result would not have been obtained if the steady-state distribution of poly(A)$^+$ RNA in *mtr1-1* at 37°C (bright nucleus, dark cytoplasm) reflected primarily an acceleration of turnover of cytoplasmic poly(A)$^+$ RNA.

After 3 hr and 24 hr at 37°C, followed by replating at 23°C, recovery of *mtr1-1* was essentially 100% and 10%, respectively. After incubation for 1–3 hr at 37°C, a heterogeneous mixture of unbudded and budded cells was seen.

Steady-State Levels, Synthesis, and Turnover of RNA. The impact of the *mtr1-1* mutation on RNA levels and turnover has been evaluated by comparison to *rpb1-1* and a *mtr1-1 rpb1-1* double mutant. In the latter two cases, upon shift to 37°C one can directly follow the turnover of preexisting—i.e., >90% cytoplasmic—mRNA (39). As illustrated in Fig. 2 *A1* and *A2*, *mtr1-1* cells at 37°C maintain ≈45% of their initial (23°C) levels of total poly(A)$^+$ RNA for 3 hr at 37°C. Northern analysis of actin mRNA shows a comparable persistence of normal-sized transcripts (not shown). For the *mtr1-1 rpb1-1* double mutant, turnover of cytoplasmic poly(A)$^+$ RNA is accelerated relative to *rpb1-1*.

Synthesis of poly(A)$^+$ RNA and synthesis and processing of rRNAs were evaluated in 2-min and 10-min $^{32}P_i$ pulse-labeling experiments, respectively. Synthesis of poly(A)$^+$ RNA and labeling of 35S, 27S, 25S, 20S, 18S, 5.8S, and 5S rRNAs and tRNA (4S) by *mtr1-1* continued at levels similar to wt after 30 min at 37°C but were severely inhibited within 90 min (not shown).

To evaluate turnover of intranuclear poly(A)$^+$ RNA, cells incubated 1–2 hr at 37°C were reincubated 0–3 hr at 37°C with thiolutin. Fig. 2*B* shows that by comparison to wt cells, turnover is severely slowed in *mtr1-1*. The slow turnover seen for *rpb1-1* (Fig. 2*B*) pertains to that poly(A)$^+$ RNA that remains after the preincubation at 37°C.

Reversibility of RNA Accumulation. When *mtr1-1* cells were incubated 3 hr at 37°C and then returned to 23°C, the *in situ* hybridization signal returned to normal over 2–3 hr (not shown). Normalization was dependent on ATP production

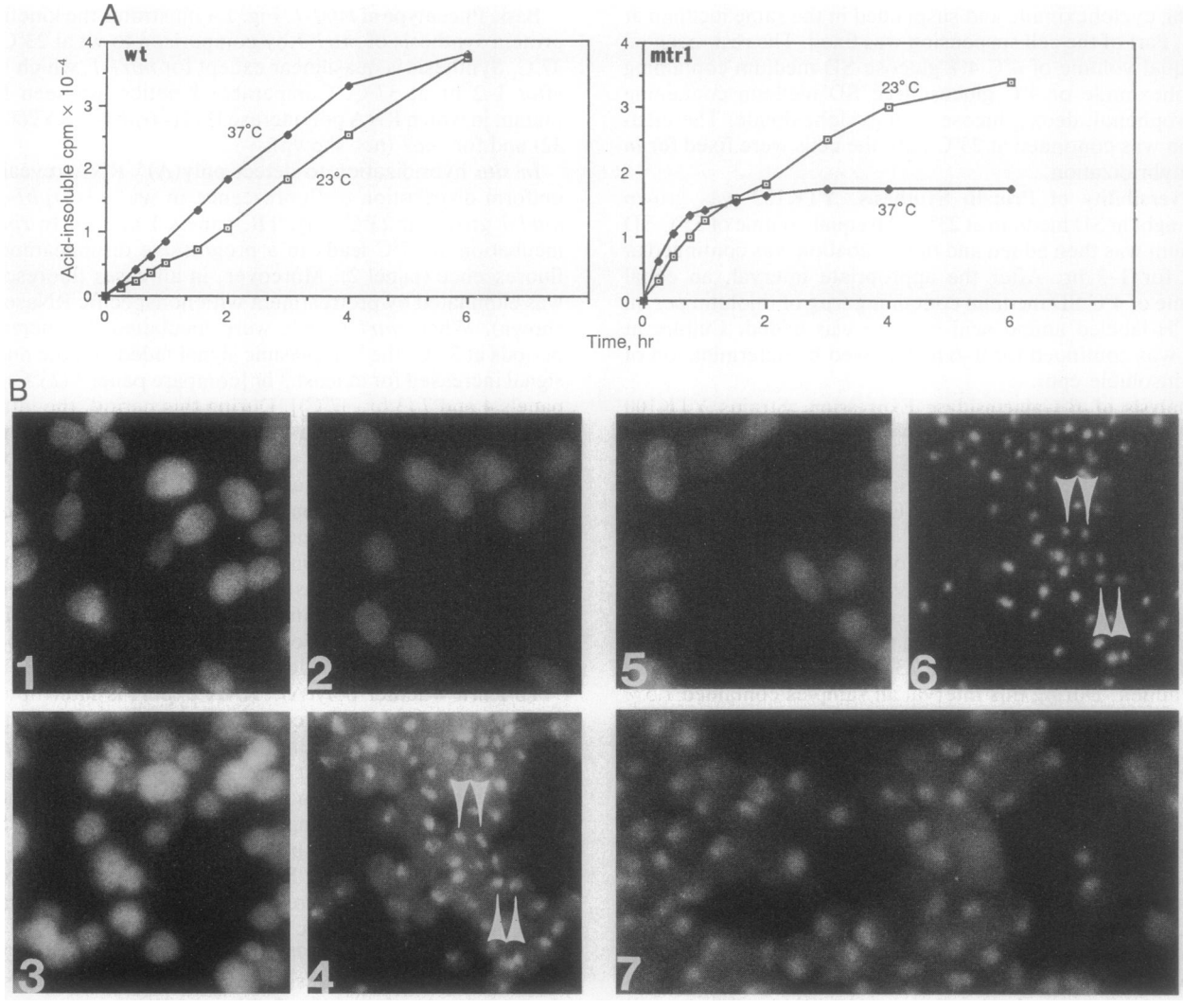

FIG. 1. (*A*) Protein synthesis in wt and *mtr1-1*. Each cell type was incubated for increasing periods of time at 23°C or 37°C in the presence of mixed ^{3}H-labeled amino acids. At the indicated times, samples were withdrawn for trichloroacetic acid precipitation. (*B*) Poly(A)$^+$ RNA distribution in a *mtr1-1, rpb1-1* or a *mtr1-1 rpb1-1* double mutant. Panel 6 is a DAPI-stained image corresponding to panel 4. Panels 1 and 2 compare the *in situ* signal in *rpb1-1* at 23°C (panel 1) with that seen after 3 hr at 37°C (panel 2). Panel 3, *mtr1-1* at 23°C. Panels 4 and 7, *mtr1-1* after 3 hr at 37°C. In panels 4 and 6, several corresponding nuclei are designated. Panel 5, *mtr1-1 rpb1-1* double mutant after 3 hr at 37°C.

(Fig. 3) but was not altered by inclusion of 100 μg of cycloheximide per ml.

To evaluate whether RNA that was accumulated in the nucleus at 37°C can pass to the cytoplasm at 23°C, *mtr1-1* cells incubated 1–3 hr at 37°C were returned to 23°C in the presence of thiolutin and mixed ^{3}H-labeled amino acids. As illustrated in Fig. 4*A*, protein synthesis does resume at 23°C. To examine reversibility of export of a single mRNA, we transformed *mtr1-1* with pLGSD5 (33), which codes for β-galactosidase under *GAL10* control. When these cells were grown in raffinose at 23°C, no enzyme activity was seen; however, growth on galactose at 23°C (but not 37°C) rapidly induced activity. Fig. 4*B* illustrates an experiment in which transformed *mtr1-1* cells were incubated 2.5 hr at 37°C in the presence of galactose or raffinose. In each case, the subsequent 23°C incubation included 1% galactose, 2% glucose, and 1.5% raffinose. For galactose (but not raffinose) a major increase in enzyme activity was seen at 23°C. Thus, most activity seen with galactose is due to transcription that occurred at 37°C. When transformed wt was exposed to galactose after 1, 2, and 3 hr at 37°C, 95, 280, and 460 units of enzyme activity were produced.

Protein Synthesis, mRNA Splicing, and the Poly(A)-Binding Protein (PABP) Are Not Critical for Export of the Average Poly(A)$^+$ RNA. Neither protein synthesis, mRNA splicing, nor a normal titer of functional PABP is essential for synthesis of most mRNAs (1, 3, 20, 25). We therefore have used *in situ* hybridization to evaluate the importance of these parameters for RNA export: when wt cells were incubated for 3 hr at 37°C in the presence of cycloheximide, the uniform fluorescent signal was more intense than in controls, probably due to stabilization of mRNA (37). Incubation for 3 hr at 37°C of a mutant that is ts for translation initiation (*ts 187*; ref. 25) or any of four ts splicing mutants (*prp2, prp5, prp8*, and *prp11*; refs. 20 and 27) did not modify the uniform *in situ* hybridization signal. The importance of the PABP for poly(A)$^+$ RNA export was evaluated by shifting a strain that harbors a ts allele of the PABP (YAS120; ref. 1) to 37°C for 3–12 hr and by shifting a strain in which expression of PABP is under galactose control (YAS352; ref. 1) to glucose medium for up to 30 hr. In neither case was an obvious change in the *in situ* hybridization pattern seen (not shown).

The *rna1* Mutant Is Distinct from *mtr1*. We have constructed heterozygous +/*mtr1-1* +/*rna1-1* diploids at 23°C and shown that they grow at 37°C. We have also inquired whether *rna1-1* gives a nuclear *in situ* hybridization signal comparable to that of *mtr1-1*. This is not the case, although some nuclear signal is seen after shorter incubation at 37°C.

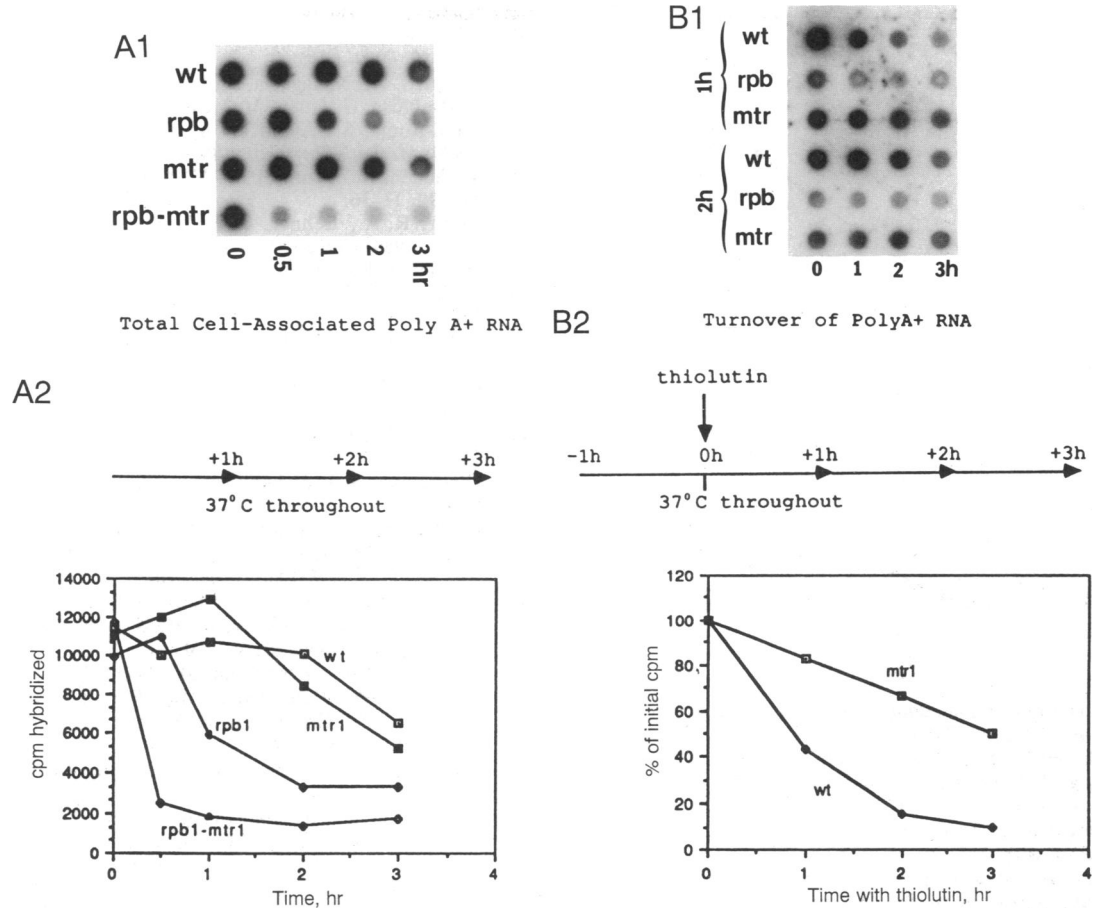

FIG. 2. (*A*) Quantitation of poly(A)$^+$ RNA. wt, *mtr1-1*, *rpb1-1*, and *mtr1-1 rpb1-1* cells were maintained for 0–3 hr at 37°C. Ten-microgram samples of total RNA were then analyzed by dot blot (*A1*) probed with ^{32}P-oligo(dT). (*A2*) Quantitation of data in *A1*. (*B*) Turnover of poly(A)$^+$ RNA in wt, *rpb1-1*, and *mtr1-1*. Cells were incubated at 37°C for 1 hr (top) or 2 hr (bottom) and then recultured with 3 μg of thiolutin per ml for 0–3 hr. Ten micrograms of total RNA from each sample was probed as in *A*. (*B2*) Quantitation of data in *B1*; 1-hr preincubation.

DISCUSSION

After polyadenylylation, the export of mRNA from the nucleus of yeast is rapid and efficient (39). By analogy to animal cells, the intranuclear pre-mRNA is likely to be bound to the karyoskeleton. Export must involve targeting to the sites of exit and translocation through them, probably as a RNA–protein complex.

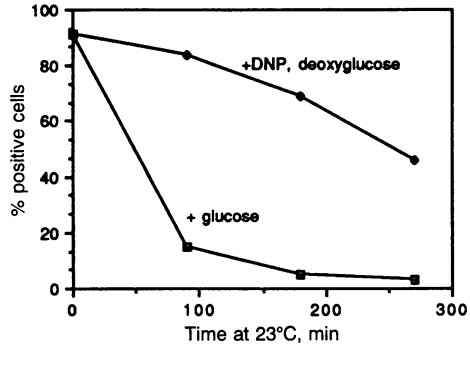

FIG. 3. Normalization of the *in situ* hybridization pattern requires ATP. *mtr1-1* cells were incubated 3 hr at 37°C and subsequently returned to 23°C for increasing periods in the absence or presence of 0.1 mM dinitrophenol and 0.5% deoxyglucose. At each time point the fraction of cells exhibiting a distinct nuclear signal was counted. Cells maintained for up to 6 hr at 37°C gave an *in situ* hybridization pattern similar to that at 3 hr.

Our strategy to identify components responsible for mRNA export would not identify lesions that block polyadenylylation, inhibit RNA polymerase II, cause rapid turnover of intranuclear mRNA, or stabilize cytoplasmic mRNA. Judging from our observations on *prp2*, *prp5*, *prp8*, and *prp11*, they also would not identify lesions that primarily block pre-mRNA splicing.

The intranuclear accumulation of poly(A)$^+$ RNA in conjunction with the persistence of intranuclear poly(A)$^+$ RNA in the absence of RNA synthesis and the data on reversibility indicate that export has indeed been interrupted at 37°C. Since the kinetics of protein synthesis in *mtr1-1* at 37°C are very similar to *rpb1-1*, the arrest of export must occur in no more than ≈10 min. We have also used subcellular fractionation to document inhibition of export of [^{3}H]uridine-labeled poly(A)$^+$ RNA in *mtr1-1* at 37°C vs. 23°C. These observations are striking: after 30 min labeling, only 2–3% of total ^{3}H-labeled poly(A)$^+$ RNA is recovered in the nuclear fraction of wt at 23°C or 37°C or *mtr1-1* at 23°C. By contrast, >40% is in the nuclear fraction of *mtr1-1* at 37°C.

The reversibility of inhibition of translation and the stability of poly(A)$^+$ RNA in *mtr1-1* show that poly(A)$^+$ RNA in the *mtr1-1* nucleus at 37°C is considerably more stable than the average intron or cytoplasmic mRNA of wt cells (19, 37).

The volume of the nucleus is only about 1/50 of the cell volume. Since the total amount of poly(A)$^+$ RNA in *mtr1-1* after several hours at 37°C is ≈1/2 that of wild type and since most of this poly(A)$^+$ RNA is in the nucleus, the intranuclear concentration of poly(A)$^+$ RNA must be much higher in

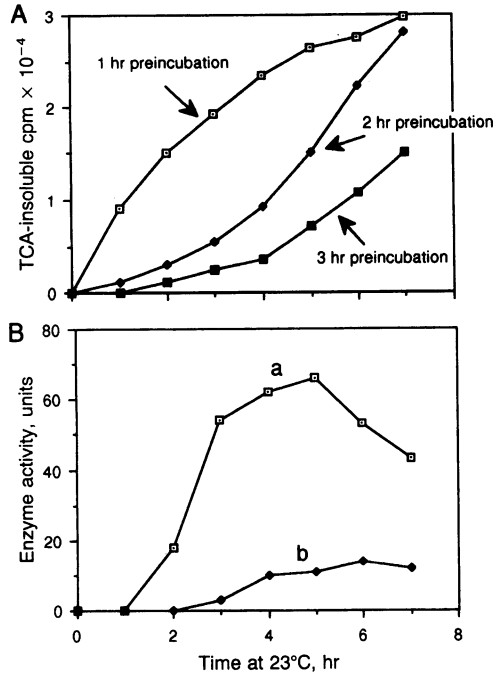

FIG. 4. Transcripts that accumulate in the nucleus of *mtrl-1* at 37°C are translated at 23°C. TCA, trichloroacetic acid. (*A*) *mtrl-1* cells were incubated 1–3 hr at 37°C and then returned to 23°C in the presence of 3 μg of thiolutin per ml and ^{3}H-labeled amino acids. Protein synthesis resumes at 23°C. (*B*) *mtrl-1* cells transformed with pLGSD5 were incubated 2.25 hr at 37°C with galactose to induce *lacZ* transcription (a) or with raffinose (b). The cells were then supplemented with glucose and normalized with regard to sugar composition to terminate transcription for 0–7 hr at 23°C. A burst of β-galactosidase activity is seen after a lag.

mtrl-1 after 3 hr at 37°C than in normal cells. This exaggeration may stop further RNA synthesis after 30 min and may contribute to the lag period that precedes translation of accumulated mRNAs once the temperature is reduced to 23°C. The lag may also reflect the progressive depletion at 37°C of factors that are essential for protein synthesis.

Why does mRNA export stop in *mtrl-1* at 37°C? Our data argue that neither the interruption of protein synthesis, inhibition of splicing, nor lesions in the PABP are responsible. The 5′ cap methylation and poly(A) length distributions of the average transcript in *mtrl-1* at 37°C are as expected (T.K., Y.Z., A.M.T., P. Narayan, and F. Rottman, unpublished). Thus, the mutation might affect a critical protein that accompanies mRNA from the nucleus to the cytoplasm. Alterations in its structure could stop export and account for accelerated mRNA turnover in the cytoplasm.

We thank S. Lemmon, P. Hieter, R. Young, A. Sachs, and L. Guarente for yeast strains and plasmids, N. Belcher for thiolutin, S. Lemmon and Y. Kitagawa for advice, S. Meyale, G. Tucker, and D. Cai for technical help, M. Ward for preparing the manuscript, and the National Science Foundation for Grant DCB90-02365 and the National Institutes of Health for Grant GM-46569.

1. Sachs, A. & Davis, R. (1989) *Cell* **58**, 857–867.
2. Ohno, M., Kataoka, N. & Shimura, Y. (1991) *Nucleic Acids Res.* **18**, 6989–6995.
3. Adam, S. A., Nakagawa, T., Swanson, M. S., Woodruff, T. K. & Dreyfuss, G. (1986) *Mol. Cell. Biol.* **6**, 2932–2943.
4. Piñol-Roma, S., Adam, S. A., Choi, Y. D. & Dreyfuss, G. (1989) *Methods Enzymol.* **180**, 410–418.
5. Verheijen, R., Venrooij, W. V. & Ramaeker, F. (1988) *J. Cell Sci.* **90**, 11–36.
6. Borer, R. A., Lehner, C. F., Eppenberger, H. M. & Nigg, E. A. (1989) *Cell* **56**, 379–390.
7. Warner, J. R. (1989) *Microbiol. Rev.* **53**, 256–271.
8. Guddat, U., Bakken, A. H. & Pieler, T. (1990) *Cell* **60**, 619–628.
9. Tobian, J. A., Drinkard, L. & Zasloff, M. (1985) *Cell* **43**, 415–422.
10. Caldwell, D. C. & Emerson, C. P., Jr. (1985) *Cell* **42**, 691–700.
11. Hamm, J. & Mattaj, I. W. (1990) *Cell* **63**, 109–118.
12. Bataillé, N., Helser, T. & Fried, H. (1990) *J. Cell Biol.* **111**, 1571–1582.
13. Dworetzky, S. I. & Feldherr, C. M. (1988) *J. Cell Biol.* **106**, 575–584.
14. Featherstone, C., Darby, M. K. & Gerace, L. (1988) *J. Cell Biol.* **107**, 1289–1297.
15. Agutter, P. S. (1988) *Subcellular Biochemistry*, ed. Roodyn, D. (Plenum, New York), Vol. 10, pp. 281–356.
16. Clawson, G. A., Feldherr, C. M. & Smuckler, E. A. (1985) *Mol. Cell. Biochem.* **67**, 87–100.
17. Schröder, H. C., Bachmann, M., Diehl-Seifert, B. & Müller, W. E. G. (1987) *Prog. Nucleic Acid Res. Mol. Biol.* **34**, 89–142.
18. Sripati, C. E., Groner, Y. & Warner, J. R. (1976) *J. Biol. Chem.* **251**, 2898–2904.
19. Vijayraghavan, U., Company, M. & Abelson, J. (1989) *Genes Dev.* **3**, 1206–1216.
20. Woolford, J. L., Jr. (1989) *Yeast* **5**, 439–457.
21. Brawerman, G. (1981) *CRC Crit. Rev. Biochem.* **10**, 1–38.
22. Allen, J. L. & Douglas, M. G. (1989) *J. Ultrastruct. Mol. Struct. Res.* **102**, 95–108.
23. Davis, L. I. & Fink, G. R. (1990) *Cell* **61**, 965–978.
24. Nehrbass, U., Kern, H., Mutvei, A., Horstmann, H., Marshallsay, B. & Hurt, E. C. (1990) *Cell* **61**, 979–989.
25. Hartwell, L. & McLaughlin, C. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 468–474.
26. Hartwell, L. H., McLaughlin, C. S. & Warner, J. R. (1970) *Mol. Gen. Genet.* **109**, 42–56.
27. Lustig, A. J., Lin, R.-J. & Abelson, J. (1986) *Cell* **47**, 953–963.
28. Shiokawa, K. & Pogo, A. O. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2658–2662.
29. Hopper, A. K., Traglia, H. M. & Dunst, R. W. (1990) *J. Cell Biol.* **111**, 309–321.
30. Sikorski, R. & Hieter, P. (1989) *Genetics* **122**, 19–27.
31. Rose, M. D., Winston, F. & Hieter, P. (1990) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
32. Nonet, M., Scafe, C., Sexton, J. & Young, R. (1987) *Mol. Cell. Biol.* **7**, 1602–1611.
33. Guarente, L., Yocum, R. R. & Gifford, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7410–7414.
34. Schmitt, M. E., Brown, T. A. & Trumpower, B. L. (1990) *Nucleic Acids Res.* **18**, 3091–3092.
35. Rubin, G. (1974) *Eur. J. Biochem.* **41**, 197–202.
36. Knapp, G., Beckmann, J. S., Johnson, P. F., Fuhrman, S. A. & Abelson, J. (1978) *Cell* **14**, 221–236.
37. Herrick, D., Parker, R. & Jacobson, A. (1990) *Mol. Cell. Biol.* **10**, 2269–2284.
38. Tipper, D. J. (1973) *J. Bacteriol.* **116**, 245–256.
39. Groner, B. & Phillips, S. L. (1975) *J. Biol. Chem.* **250**, 5640–5646.