MOLECULAR AND CELLULAR BIOLOGY, Sept. 1996, p. 5139–5146 Vol. 16, No. 9
0270-7306/96/$04.00+0
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A DEAD-Box-Family Protein Is Required for Nucleocytoplasmic Transport of Yeast mRNA

SHUANG LIANG, MIDORI HITOMI, YAN-HUI HU,† YAN LIU, AND ALAN M. TARTAKOFF*

Institute of Pathology and Cell Biology Program, Case Western Reserve University, Cleveland, Ohio 44106

Received 9 April 1996/Returned for modification 10 May 1996/Accepted 3 June 1996

An enormous variety of primary and secondary mRNA structures are compatible with export from the nucleus to the cytoplasm. Therefore, there seems to be a mechanism for RNA export which is independent of sequence recognition. There nevertheless is likely to be some relatively uniform mechanism which allows transcripts to be packaged as ribonucleoprotein particles, to gain access to the periphery of the nucleus and ultimately to translocate across nuclear pores. To study these events, we and others have generated temperature-sensitive recessive mRNA transport (*mtr*) mutants of *Saccharomyces cerevisiae* which accumulate poly(A)$^+$ RNA in the nucleus at 37°C. Several of the corresponding genes have been cloned. Upon depletion of one of these proteins, Mtr4p, conspicuous amounts of nuclear poly(A)$^+$ RNA accumulate in association with the nucleolus. Corresponding dense material is also seen by electron microscopy. *MTR4* is essential for growth and encodes a novel nuclear protein with a size of ~120 kDa. Mtr4p shares characteristic motifs with DEAD-box RNA helicases and associates with RNA. It therefore may well affect RNA conformation. It shows extensive homology to a human predicted gene product and the yeast antiviral protein Ski2p. Critical residues of Mtr4p, including the *mtr4-1* point mutation, have been identified. Mtr4p may serve as a chaperone which translocates or normalizes the structure of mRNAs in preparation for export.

The mechanism of export of mRNA from the nucleus to the cytoplasm is remarkably accommodating in the sense that an enormous variety of primary and secondary RNA structures are compatible with export. The fact that neither the 5′ methyl cap structure nor the 3′ poly(A) tail appears altogether essential for export (16) suggests that there is a mechanism in the nucleus which allows for RNA recognition independent of the sequence. It is likely that during or immediately after their synthesis, transcripts are quickly packaged into ribonucleoprotein particles (RNP) which enable them to gain access to the periphery of the nucleoplasm and interact specifically with components of nuclear pores, through which they subsequently translocate (14, 42). If this itinerary is relatively uniform for most mRNAs, it is reasonable to postulate that there are factors which serve as RNA chaperones and/or normalize RNA secondary structure. Single-stranded RNAs spontaneously acquire an extensive secondary structure, which is subject to proteins with annealing activity (p53 and heterogeneous nuclear RNP [hnRNP] A1 [24, 25]) and melting activity (helicases [34]). Studies of the yeast *Saccharomyces cerevisiae* have identified several proteins which, when mutated, lead to nuclear accumulation of poly(A)$^+$ RNA without inhibiting pre-mRNA splicing. Apart from nucleoporins (8), these includes components of a nucleocytoplasmic GTPase cycle [the Cnr1/2p (Gsp1/2p) GTPases, Mtr1p (Prp20p), and Rna1p (6, 35)], a cytoplasmic protein (Mtr7p [34a]), the nucleolar protein Mtr3p (19), the nuclear proteins Mtr2p (19) and Rat1p (1), and the nuclear hnRNP-like protein Mtr13p/Nab1p/Npl3p/Nop3p (4, 31, 39).

In the present report we further investigate a yeast temperature-sensitive (ts) *mtr4-1* mutant, which accumulates poly(A)$^+$ RNA in the nucleus after incubation at 37°C (18). The cloning of the corresponding gene identifies its product as a DEAD-box putative RNA helicase.

* Corresponding author. Phone: (216) 368-5544. Fax: (216) 368-5484.

† Present address: Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.

MATERIALS AND METHODS

The yeast strains used in this study are described in Table 1. The original *mtr4-1* mutant was backcrossed once with BJ2698 and once with BJ3502 (17). Both YSL401 and YSL402, derived from the second backcross, showed 2:2 cosegregation of ts growth and nuclear accumulation of poly(A)$^+$ RNA upon incubation at 37°C. Yeast cells were grown either in complete medium (YPD or YPGal) or in synthetic dropout (SD) medium. Diploid yeast cells were sporulated in liquid YEPA and then transferred to liquid SPM medium, and yeast transformations were carried out by a modified lithium acetate method (29, 33, 38). Double mutants involving *mtr4-1* were constructed either by performing a genetic cross between *mtr4-1* and other single mutants (*mtrX*) followed by tetrad dissection or by a one-step integration of pIM(ts)Δpro (Table 2) digested by *Bam*HI into the *MTR4* locus of single *mtr* mutants other than *mtr4-1*. The latter step resulted in both a nonfunctional and a ts version of *MTR4* in a haploid *mtrX* background. None of the double mutants involving *mtr4-1* grew at 37°C when backcrossed to *mtr4-1* or to *mtrX*.

The plasmids used in this study are listed in Table 2. The low-copy-number plasmid pRM contains the full-length *MTR4* gene. The nonreplicative plasmid pIM was used to confirm the identity of *MTR4* by integration. pRMts contains the *mtr4-1* ts mutation rescued onto the pRM plasmid by gap repair (see below). Plasmid pFL435L was used to create an inducible conditional mutant by substituting a *GAL10* promoter for the authentic *MTR4* promoter in a haploid strain. This plasmid was constructed in several steps: first, a 800-bp *Cla*I-*Ase*I fragment containing the 5′ noncoding sequence of *MTR4* was ligated adjacent to the end of the *TRP1* marker in pFL35 (3); a 700-bp *Eco*RI-*Bam*HI fragment containing the *GAL1-GAL10* promoter was filled in with Klenow enzyme and inserted into the *Pvu*II site of the resulting plasmid so that the *GAL10* promoter was adjacent to the multicloning site of the plasmid. Finally, a PCR-amplified 3.7-kb fragment containing the entire open reading frame (ORF) and the 3′ end of *MTR4* was inserted at the *Eco*RI site so that the ATG initiation codon of *MTR4* was about 100 bp from the *GAL10* promoter. pY*SKI2* (44) and pY*hORF2* were high-copy-number plasmids used for overexpression of the yeast *SKI2* gene or human *ORF2* cDNA with an *ADH* promoter (Table 2). pY*hORF2* was constructed by replacing *SKI2* in pY*SKI2* with human cDNA.

Cloning. A YCp50-based genomic library (29) was introduced into *mtr4-1* (YSL401), and plasmids were isolated from colonies which grew at 37°C on plates with uracil-dropout medium. Each isolated plasmid was reintroduced separately into the mutant, and the growth of transformants was verified at 37°C. The complementing activity was localized by restriction mapping, serial deletion mapping, transposon insertion mapping (2), and finally by *Exo*III unidirectional mapping. The complementing gene was demonstrated to map to the ts locus by

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
YSL401(T188)	*MAT***a** *mtr4-1 ura3-52 lys2-801 ade2-101 his3-Δ200 pep4::HIS3 prb1-Δ1.6R GAL*$^+$	This study
YSL402(T189)	*MAT*α *mtr4-1 ura3-52 lys2-801 pep4::HIS3 prb1-Δ1.6R GAL*$^+$	This study
YSL2589[a]	*MAT***a**/α *ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 GAL*$^+$ / *ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 GAL*$^+$	This study
W303	*MAT*α *ura3-52 ade2-1 trp1-1 leu2-3,112 his3-11,15 GAL*$^+$	B. Futcher
YSL303	*MAT*α *TRP::GAL10-MTR4 ura3-52 ade2-1 trp1-1 leu2-3,112 his3-11,15 GAL*$^+$	This study
CRL 2101	*MAT*α *prp2-1 ade2 his3 lys2-801*	R.-J. Lin
prp5-3a	*MAT***a** *prp5-3 ade2 ura3-52 his3 leu2-3,112 tyr1*	J. Abelson
prp16-2α	*MAT*α *prp16-2*	C. Guthrie
prp22:9-6c	*MAT***a** *prp22 ade2-101 his3J200 ura3-52 lys2-801*	J. Abelson
prp28-1α	*MAT*α *prp28-1 his4 trp1*	C. Guthrie
YAS165	*MAT***a** *spb4-1 his3 leu2 trp1*	A. Sachs
YTK206	*MAT*α *ura3-52 leu2-Δ1 ΔMTR2::LEU2* [pTK204]	19

[a] This strain results from a cross between YPH258 and YPH259 (38).

linearizing an integrative *MTR4 URA3* plasmid (pIM) within the *MTR4* coding sequence and transforming it into a wild-type strain (W303; TS$^+$ *ura3*). The transformant was crossed to YSL401 (*mtr4-1 ura3*), and the resulting diploid was sporulated. TS$^+$ and URA$^+$ cosegregated in all eight complete tetrads. To identify the mutation in *mtr4-1*, pRM was digested by *Xba*I-*Pfl*MI, *Bsp*EI-*Bsp*EI, *Pfl*MI-*Bst*EII, *Bst*EII-*Hin*dIII, and *Bam*HI-*Hin*dIII. The gapped plasmids were gel purified and transformed into *mtr4-1* (YSL401). Transformants were grown at 25°C on plates with uracil-dropout medium. Replicative plasmids were isolated from these transformants and reintroduced into YSL401. The growth of each transformant at 25°C was compared with that at 37°C.

Disruption of *MTR4*. An internal *Xba*I-*Aat*II fragment of *MTR4* was deleted and replaced by a *LEU2* marker, and the resulting plasmid, pDM, was linearized by *Bam*HI-*Hin*dIII digestion and introduced into the diploid strain YPH2589. After selective growth on plates with leucine-dropout medium, disruption of the *MTR4* gene in the transformants was verified by Southern blotting. One transformant was sporulated and tetrad dissected to verify the growth of spores at 25°C.

Plasmid constructions for structural studies of Mtr4p. A sequence overlapping both the ATG codon and the *Xba*I site (residues 548 to 858) of *MTR4* was PCR amplified with pRMBg as the template. This fragment was fused to a *GAL10*/*CYC1* promoter which had been subcloned separately into pRS316. The promoter-containing fragment of *MTR4* in pRM was replaced by fragments containing the *GAL10*/*CYC1*-PCR to generate pGM400. pGM410 contains a hemagglutinin (HA) tag at the *Bam*HI site. pGM420 contains an HA tag at the *Bgl*II site. In-frame deletions were created by removing restriction fragments from these plasmids (Table 2). Site-directed mutations were introduced into single- or double-stranded pRM(B)HA DNAs by a megaprime-PCR mutagenesis technique or by using the Sculptor mutagenesis kit (Amersham, Inc.) with mutagenic oligonucleotides. DNA sequencing was performed to verify all fragments containing the mutations.

TABLE 2. Plasmids used in this study[a]

Plasmid	Description
pRM	A 4-kb fragment containing the entire *MTR4* gene subcloned into pRS316[b]
pIM	Similar to pRM except that the vector is pRS306[b]
pDM	A *LEU2* marker from pRS305[b] was substituted for an *Xba*I-*Aat*II fragment of *MTR4* in pIM
pRM(ts)	This plasmid contains the *mtr4-1* mutation rescued by gap repair
pIM(ts)Δpro	An *Xba*I-*Hin*dIII fragment from pRM9(ts) was subcloned into pIM and digested with *Spe*I-*Hin*dIII to create a promoterless integrative form of *mtr4-1* for double mutant construction
pRMBg	A *Bgl*II site was created by changing T-12 to A in pRM
pRrM(Bg)HA	A sequence coding for the influenza virus HA epitope[c] was inserted into the *Bgl*II site of pRMBg
pRM(B)HA	Identical to pRM(Bg)HA but insertion was at the *Bam*HI site of pRM(ts)
pRM(ts)	Identical to pRM(B)HA but insertion was at the *Bam*HI site of pRM(ts)
pGM435L	A pFL35[d]-based plasmid containing the 5′ nontranscribed sequence of *MTR4-TRP1-GAL10-ORF* and 3′ untranslated region of *MTR4*
pYSKI2	This plasmid, which contains an *ADH* promoter, *SKI2* gene, *URA3* marker, and 2μm replicon, was produced by replacing the *TRP1* marker of pYeFlag SKI2[e] with a *URA3* marker from pFL44L[d]
pYhORF2	The human cDNA for *ORF2*[f] was substituted for the *SKI2* gene in pYSKI2
pGM411	A *Bgl*II-*Xba*I fragment was removed from pGM410[g]
pGM412	A *Bgl*II-*Pfl*MI fragment was removed from pGM410
pGM421	A *Bsp*EI-*Bsp*EI fragment was removed from pGM420
pGM422	A *Bst*XI-*Bst*XI fragment was removed from pGM420
pGM423	A *Bst*EII-*Hin*dIII fragment was removed from pGM420
pGM424	A *Bsp*EI-*Hin*dIII fragment was removed from pGM420

[a] This study was the source for each of these plasmids.
[b] R. S. Sikorski and P. Hieter (38).
[c] See Materials and Methods.
[d] N. Bonneaud et al. (3).
[e] W. R. Widner and R. B. Wickner (44).
[f] N. Nomura et al. (24a); GenBank accession no. D29641.
[g] Both pGM410 and pGM420 express *MTR4* from a *GAL10*/*CYC1* promoter (see Materials and Methods).

Epitope tagging of Mtr4p. A double-stranded synthetic oligonucleotide sequence coding for the influenza virus HA tag (YPYDVPDYA) with compatible ends was inserted in frame into either the *Bgl*II site of pRMBg or the *Bam*HI site of pRM (see above) to give rise to pRM(Bg)HA or pRM(B)HA, respectively. The *Bgl*II site is 12 bp downstream of the ATG codon (created by site-directed mutagenesis), and the *Bam*HI site is 795 bp upstream of the stop codon.

In situ hybridization, indirect immunofluorescence, and electron microscopy. In situ hybridization, indirect fluorescence, and electron microscopy were done as described previously (18). For double detection of both poly(A)$^+$ RNA and yeast antigens, cells were incubated for 2 h each with a primary antibody followed by a secondary antibody diluted in TBSG buffer (20 mM Tris-HCl, 137 mM NaCl, 3 mM KCl, 1.5 mM $MgCl_2$, 5 mg of bovine serum albumin per ml, 1 mg of glycine per ml, 0.05% sodium azide [pH 7.6]) supplemented with 5 mM heparin. Samples were washed with TBSG buffer five times for 5 min after each incubation. The biotinylated oligo(dT) probe was then added, and processing was continued for in situ hybridization. The mouse monoclonal antibodies 12CA5 (from D. Templeton), A66, anti-NOP1p (from J. Aris), anti-β-galactosidase (Promega), and the rabbit polyclonal anti-NSP1 affinity-purified antibody EC10 (from E. Hurt) were used at 1:100, 1:5, 1:1,000, and 1:5 dilutions in TBSG, respectively. The fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G and the rhodamine-conjugated goat anti-rabbit immunoglobulin G were used at 1:100 to 1:200 dilutions (Jackson Laboratory).

Depletion of MTR4 expression. The plasmid pFL435L (Table 2) was linearized by *Cla*I-*Aat*II digestion. The gel-purified fragment containing the 5′ end of *MTR4-TRP1-GAL10-MTR4* ORF plus the 3′ sequence was introduced into the haploid strain W303 and selected for growth on galactose-containing SD medium without tryptophan. Of 24 transformants tested, 20 grew only in medium containing 2% galactose or 2% raffinose plus 0.1% galactose. One such strain was named YSL303. For Mtr4p depletion, cells were shifted from galactose-containing medium to glucose-containing medium.

Extraction of HA-tagged Mtr4p. The conditional strain *GAL10-MTR4*(YSL303) transformed with pRM(B)HA was used. As a negative control, pRM was used in place of pRM(B)HA. Five A_{260} units of early-log-phase cells was washed in water and in standard extraction buffer A (10 mM NaCl, 20 mM Tris-HCl [pH 7.4], 2 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and aprotinin, pepstatin, and leupeptin at 5 μg/ml each). The pellets were vortexed in 300 μl of the same buffer with 100-μl glass beads (0.45-mm diameter; Sigma) seven times for 30 s each with 30-s intervals on ice. Cell debris was removed by centrifugation twice for 10 min each at 15,000 rpm in a Tomy microcentrifuge. The supernatants were transferred to new tubes and analyzed in parallel with the pellets after the addition of reducing Laemmli buffer containing sodium dodecyl sulfate to extract proteins from the pellets.

Glycerol gradient fractionation. Samples (300 μl) of the cell extract were loaded onto 10 to 30% glycerol gradients in extraction buffer A and sedimented at 35,000 rpm for 24 h at 4°C in a Beckman SW41 rotor. Twenty-five 470-μl fractions were collected with a gradient fractionator (Auto Densi-Flow II C; Haake Buchler). Molecular standards (yeast alcohol dehydrogenase [150 kDa] and apoferritin [443 kDa]) were mixed with equal volumes of 2× extraction buffer A and centrifuged in parallel gradients under the same conditions. The position of each standard in the gradient was determined by measuring the optical density at 280 nm.

RESULTS

Cloning of *MTR4* and overall structure of Mtr4p. Pre-mRNA splicing, rRNA processing, and tRNA processing appear normal in an *mtr4-1* mutant; however, the average length of the poly(A) tail increases and small amounts of oversized *CRY1* transcripts can be detected (18). We transformed an *mtr4-1* strain with a YCp50-based genomic library to clone the corresponding gene (Fig. 1A). Plasmids which complement the growth defect were analyzed in detail and subsequently integrated at the corresponding locus to prove that the cloned gene was not an extragenic suppressor (see Materials and Methods). The *MTR4* gene encodes a novel protein of 1,073 amino acids (Fig. 1B). Judging from the analysis of sporulation of a diploid with one disrupted *MTR4* gene and the arrest of growth seen in haploid strains upon termination of transcription of *MTR4* (see below), this gene is essential.

Most strikingly, Mtr4p is a member of the DEAD-box ATP-dependent helicase superfamily. Thus, it includes a characteristic set of seven consensus motifs (34) but differs from the DEAD-box prototype, eIF4A, in having AHTSAGKT in place of AXXXGKT, PIKALS versus PTRELA, TGDI versus TPGR, DEVH (also found in the NPH-2 transcription factor of vaccinia virus [37]) versus DEAD, RRGIG versus ARGXG, and GRAGRRGL versus HRIGRXXR. Like all DEAD and DEAH proteins, it includes a SAT sequence after the DEVH motif.

Comparison of the entire protein sequence shows that it resembles the sequences of several proteins with variants of the DEAD sequence (Fig. 1C), especially the yeast Ski2 protein (36% identity [44]) and predicted proteins, e.g., the human ORF2 (79% identity; GenBank accession no. D29641, after correction of the sequence by insertion of one base to yield an ORF of 1,043 rather than ~840 amino acids). Strikingly, in addition to extended regions of identity, all three proteins terminate with the sequence (K/L)I(K/H)RDIV(F/S)A(A/G)SLYL (Fig. 1B). Other related human putative ATP-dependent RNA helicases are Ski2W and the gene 170 product (7, 22). There are no obvious membrane-spanning regions or motifs characteristic of nucleoporins (FG rich), nucleolar proteins (GAR domain), or SR proteins in Mtr4p.

At the DNA level, a region rich in T is found in the putative promoter region. The AATAAA sequence located downstream of the stop codon is likely to play the role of positioning element for 3′ end formation (12, 43).

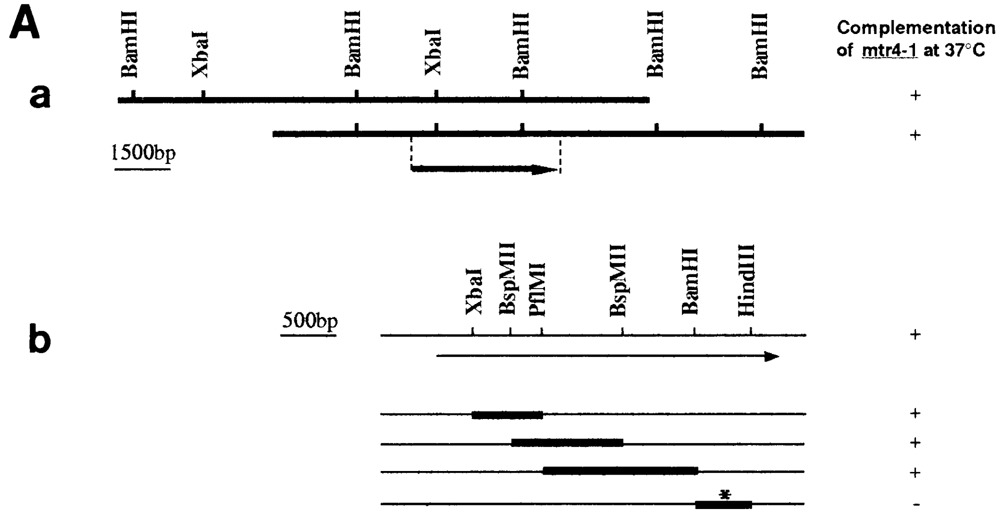
Localization of Mtr4p. To make it possible to express alternate forms of Mtr4p, we constructed a strain in which transcription of the wild-type *MTR4* gene is driven by a *GAL10* promoter (YSL303) and therefore can be terminated by a shift to glucose-containing medium. Two in-frame insertions of an HA tag into Mtr4p (either 12 bp downstream of the start codon or 795 bp upstream of the stop codon) were functional when driven by an *MTR4* promoter in YSL303, judging from their ability to rescue growth of YSL303 in glucose medium at 30°C. They also rescued growth of the *mtr4-1* strain at 37°C. HA-Mtr4p was detectable in the nucleus by immunofluorescence (Fig. 1D) and had a distribution similar to that of DNA stained with DAPI (4′,6-diamidino-2-phenylindole). It is certainly not restricted to the nucleolar crescent or the nuclear periphery.

Structure of Mtr4p. The short sequence between amino acids 855 to 869 (RKHDLHEDMKQLKRK) resembles bipartite nuclear localization signals (NLS). An arginine/lysine-rich region (residues 883 to 891) (RRRKRVLRR) was also found 14 amino acids downstream of this sequence. Fusion of residues 852 to 893 to the N terminus of β-galactosidase is sufficient to redirect β-galactosidase to the nucleus (Fig. 1E). Considering that HA epitope-tagged Mtr4p lacking residues 864 to 870 also localizes to the nucleus (data not shown), the arginine/lysine-rich region (residues 883 to 891) appears to function as an NLS. It remains to be established whether this is the only functional NLS in Mtr4p.

By use of a set of gapped plasmids, we showed that the region of Mtr4p which includes the ts mutation in *mtr4-1* is near the carboxy terminus. A point mutation (C942Y) was then identified by sequencing this region.

Site-directed mutagenesis experiments made use of the *GAL10-MTR4* strain and deletion strategy described above. These experiments show that although the DEVH sequence can be replaced by DEAD, the SAT motif (essential for RNA helicase activity [27]) cannot be replaced by LAT, the GRAGRRG motif (implicated in RNA binding [26]) cannot be replaced by GPAGRRG, and a short stretch (HEDMKQLKRKIS) near the NLS sequence cannot be shortened to HEDMKNS. Surprisingly, the conserved C-terminal SLYL tetrapeptide is not essential.

Site of accumulation of poly(A)$^+$ RNA upon depletion of Mtr4p. As detected by fluorescent in situ hybridization, poly(A)$^+$ RNA accumulates as a small spot in the nucleus of *mtr4-1* at 37°C (18). Moreover, when the *GAL10-MTR4* strain (YSL303) was shifted to glucose-containing medium for 10 to 20 h, we detected a strong nuclear signal (Fig. 2A). Double staining has been used to localize the accumulated poly(A)$^+$



B

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Mtr4p      1 M-D-----ST D-LF-DVFEE T---PVELPT DSNGEKNA-- DTNVGDTPDH TQDK--KHGL EEEKEEHEEN NSENKKIKSN KSKTEDKN-K K-------VV   78
Horf2p     1 MADAFG---- DELF-SVFEG DST------T AAGTKKDKEK DKGKWKGPPG SADKAGKR-F DGKLQSESTN NGKNKRDVDF EGTDEPIFGK KPRIEESITE   88
Ski2p      1 MSEGFSSSSI QELYQSLKEI TNNADVELFE DRITKLDFES TDEPKHANDI IKD-RFLRPS NALPWSLLDM ----VQDVPH TSSPEDCSGK LDYKELLKVP   95

Mtr4p     79 VPVLADS--- -------FEQ EASREVD-AS KGLTNSETLQ VE------QD GKVRLS-HQ- ---------- ---------- ---------- ----------  118
Horf2p    89 DLSLAD---- ---------- ------LMPR VKVQSVET-- VEG------- ---------- ---------- ---------- ---------- ----------  109
Ski2p     96 DPINRTSYQF KRTGLEGKIS GYKEEVDLKE VANANASNSL SITRSINHNQ NSVRGSTAQL PFTPGGIPMK SVKTDSEQNG SSTMANATKL LHKDGQGLFD  195

Mtr4p        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Horf2p       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ski2p    196 IPEGMNRGIK PMDSPAENED QNGQFKELKQ LNEIDNELDI RIEANEAKLK EEEKSAKSIS EEIMEEATEE TTADNADDAE IDELLPIGID FGRTKPVSKS  295

Mtr4p    119 VRHQVALPPN YDYTPIAETK -RV--NEART YPFTLDPFQD TAISCIDRGE SVLVSAHTSA GKTVVAEYAI AQSLKNKQRV IYTSPIKALS NQKYRELLAE  215
Horf2p   110 CTHEVALPAE EDYLPL---K PRV-GKAAKE YPFILDAFQR EAIQCVDNNQ SVLVSAHTSA GKTVCAEYAI ALALREKQRV IFTSPIKALS NQKYREMYEE  205
Ski2p    296 VPVKKEWAHV VDLNHKIENF DELIPNPARS CPFELDTFQK EAVYHLEQGD SVFVAAHTSA GKTVVAEYAI AMAHRNMTKT IYTSPIKALS NQKFRDFKET  395

Mtr4p    216 FGDV--GLMT GDITINPDAG CLVMTTEILR SMLYRGSEVM REVAWVIFDE VHYMRDKERG VVWEETIILL PDKVRYVFLS ATIPNAMEFA EWICKIHSQP  313
Horf2p   206 FQDV--GLMT GDVTINPTAS CLVMTTEILR SMLYRGSEVM REVAWVIFDE IHYMRDSERG VVWEETIILL PDNVHYVFLS ATIPNARQFA EWICHLHKQP  303
Ski2p    396 FDDVNIGLIT GDVQINPDAN CLIMTTEILR SMLYRGADLI RDVEFVIFDE VHYVNDQDRG VVWEEVIIML PQHVKFILLS ATVPNTYEFA NWIGRTKQKN  495

Mtr4p    314 CHIVYTNFRP TPLQHYLFPA HGDGIYLVVD EKSTFREENF QKAMASISNQ --IGDDPNST DSRGKKGQTY KGGS------ ---------- ----------  385
Horf2p   304 CHVIYTDYRP TPLQHYIFPA GGDGLHLVVD ENGDFREDNF NTAMQVLRD- -A-GDL-AKG DQKGRKGGT- KGPS------ ---------- ----------  372
Ski2p    496 IYVISTPKRP VPLEINI-WA KK-ELIPVIN QNSEFLEANF RKHKEILNGE SAKG-APSKT D-NGRGGSTA RGGRGGSNTR DGRGGRGNST RGGANRGGSR  591

Mtr4p    386 ---AKGDAK- ------GDIY K----IVKMI WKKKYNPVIV FSFSKRDCEE LALKMSKLDF NSDDEKEALT KIFNNAIALL PETDRELPQI KHILPLLRRG  471
Horf2p   373 ---------- -------NVF K----IVKMI MERNFQPVII FSFSKKDCEA YALQMTKLDF NTDEEKKMVE EVFSNAIDCL SDEDKKLPQV EHVLPLLKRG  451
Ski2p    592 GAGAIGSNKR KFFTQDGPSK KTWPEIVNYL RKRELLPMVV FVFSKKRCEE YADWLEGINF CNNKEKSQIH MFIEKSITRL KKEDRDLPQI LKTRSLLERG  691

Mtr4p    472 IGIHHSGLLP ILKEVIEILF QEGLLKVLFA TETFSIGLNM PAKTVVFTSV RKWDGQQFRW VSGGEYIQMS GRAGRRGLDD RGIVIMMIDE KMEPQ-VA--  568
Horf2p   452 IGIHHGGLLP ILKETIEILF SEGLIKALFA TETFAMGINM PARTVLFTNA RKFDGKDFRW ISSGEYIQMS GRAGRRGMDD RGIVILMVDE KMSPT-I--G  548
Ski2p    692 IAVHHGGLLP IVKELIEILF SKGFIKVLFA TETFAMGLNL PTRTVIFSSI RKHDGNGLRE LTPGE-FTLA GRAGRRGLDS TGTVIVMAYN --SPLSIATF  788

Mtr4p    569 KGMVKGQADR LDSAFHLGYN MILNLMRVEG ISPEFMLEHS FFQFQNVISV P-VMEKKLAE LKKDFDGIEV -EDEENV-KE YHEIEQAIKG YRE---DVRQ  662
Horf2p   549 KQLLKGSADP LNSAFHLTYN MVLNLLRVEE INPEYMLEKS FYQFQHYRAI PGVVEK--VK NSEEQYNKIV IPNEESVVIY YKIRQQLAKL GKE----IEE  642
Ski2p    789 KEVTMGVPTR LQSQFRLTYN MILNLLRIEA LRVEEMIKYS FSENAKETLQ PEH-EKQIKV LQEELQTIEY KSCEICDNDI EKFLE-LMLA YKEATVNLMQ  886

Mtr4p    663 VVTHPANALS FLQPGRLVEI SVNGKDNYGW GAVVDFAKRI NKRNPSAVYT DHESYIVNVV VNTMYIDSPV NLLKPFNPTL PEGIRPAE-- --EGEKSICA  758
Horf2p   643 YIHKPKYCLP FLQPGRLVKV KNEGDD-FGW GVVVNFSKK- -----SNV-K PN-S--GELD PLYVV--EVL LR--CSKESL KNSATEAAKP AKPDEKGEMQ  727
Ski2p    887 EMVKSPSILH ILKEGRLVAF RDPNDCLK-L GFVFKVSLKD AVCVIMTFTK PYKLPNGEPN HLIYFPKADG YRRRNFPKFQ KTDFYMEEVP VTAIEVITKR  985

Mtr4p    759 VIPITLDSIK SIGNLRLYMP KDIRASGQKE TVGKSLREVN RRFPDGIPV- LDPVKNMKIE DEDFLKLMKK IDVLNTK--L SSNPLTNSMR LEELYGKYSR  855
Horf2p   728 VVPVLVHLLS AISSVRLYIP KDLRPVDNRQ SVLKSIQEVQ KRFPDGIP-L LDPIDDMGIQ DQGLKKVIQK VEAFE-H-RM YSHPLHNDPN LETVYTLCEK  824
Ski2p    986 KFAAPLGKVI KKDVAALNEF NAETNNILDG KTLKEAINIE KQGLKIHQIL NDRTN---IR DEIEKLKSIK CPNLSQHIVP KFKAHVIKKK IEELYHLMSD 1082

Mtr4p    856 K-HDLHEDMK QLKRKISESQ AVIQLDDLRR RKRVLRRLGF CTPNDIIELK GRVACEISSG DELLLTELIF NGNFNEL-KP EQAAALLSCF AFQE-RCKEA  952
Horf2p   825 KSTDCNRYFK SAKRELKKAR TVLQMDELKC RKRVLRRLGF ATSSDVIEMK GRVACEISSA DELLLTEMMF NGLFNDL-SA EQATALLSCF VFQE-NSSEM  922
Ski2p   1083 QNLSL----- ---------- ----LPDYEK RLAVLKDTEF IDQNHNVLLK GRVACEINSG YELVLTELI- LDNFLGSFEP EEIVALLSVF VYEGKTREEE 1162

Mtr4p    953 PRL-KPELAE PLKAMREIAA KIAKIMKDSK IE--VVEKDY VESFRHELME VVYEWCRGAT FTQICKMTDV YEGSLIRMFK RLEELVKELV DVANTIGNSS 1049
Horf2p   923 PKL-TEQLAG PLRQMQECAK RIAKVSAEAK LEI--DEETY LSSFKPHLMD VVYTWATGAT FAHICKMTDV FEGSIIRCMR RLEELLRQMC QAAKAIGNTE 1019
Ski2p   1163 PPIVTPRLAK GKQRIEEIYK KMLCVFNTHQ IPLTQDEAEF LDRKRFAMMN VVYEWARGLS FKEIMEMSPE AEGTVVRVIT WLDEICREVK TASIIIGNST 1262

Mtr4p   1050 LKEKMEAVLK LIHRDIVSAG SLYL.....1073
Horf2p  1020 LENKFAEGIT KIKRDIVFAA SLYL.....1043
Ski2p   1263 LHMKMSRAQE LIKRDIVFAA SLYL.....1286
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FIG. 1. (A) Cloning of *MTR4* and localization of mutation in *mtr4-1*. (a) Two genomic DNA inserts obtained in the initial complementation experiment. The complementation activity was localized as indicated by the 4-kb arrow (third line). (b) Plasmid pRM, including the intact *MTR4* gene (arrow), was digested to generate a set of gaps (thick bars). The linear plasmids were used for gap repair by homologous recombination in *mtr4-1*. The growth of the transformants was determined at 37°C. Only one gap (indicated by an asterisk) did not yield TS^+ transformants. (B) Sequence of Mtr4p compared with the sequences of human ORF2 (Horf2p) and yeast Ski2p. The alignment was done with MacDNAsis software (Hitachi) and finalized by inspection. Identical residues are shaded. Underlined residues are motifs found in the DEAD-box superfamily. Dashed lines indicate the regions in Mtr4p which include a functional NLS that is rich in lysine and arginine. Sites of insertion of the HA epitope are indicated by arrowheads. The bullet indicates the site of mutation in *mtr4-1*, C942Y. The sequence can be accessed in the PIR data bank as >PIR//S56822 SKI2 protein homolog YJL050w. (C) Dendrogram illustrating the relationship of Mtr4p to other DEAD-box proteins. Note that none of the nearest relations includes the sequence DEAD. Drosophila, *Drosophila melanogaster*; C. elegans, *Caenorhabditis elegans*; Vaccinia, vaccinia virus; E. coli, *Escherichia coli*; Sulfolobus, *Sulfolobus* sp. (D) Immunofluorescence detection of Mtr4p, a nuclear protein. Mtr4p was tagged with an HA epitope, and its localization was monitored with the 12CA5 monoclonal antibody in YSL303 upon Mtr4p depletion (growth in glucose). In the control panels (−HA), no epitope was present. DAPI was used to stain the DNA. (E) Identification of an NLS. The indicated coding sequence (residues 852 to 893) was PCR amplified and fused to the N terminus of the *lacZ* gene under the control of a *GAL10*/*CYC1*-inducible promoter. After growth in galactose, cells stained with the monoclonal anti-β-galactosidase antibody gave a nuclear signal, unlike controls lacking the insert. DNA was stained with DAPI.

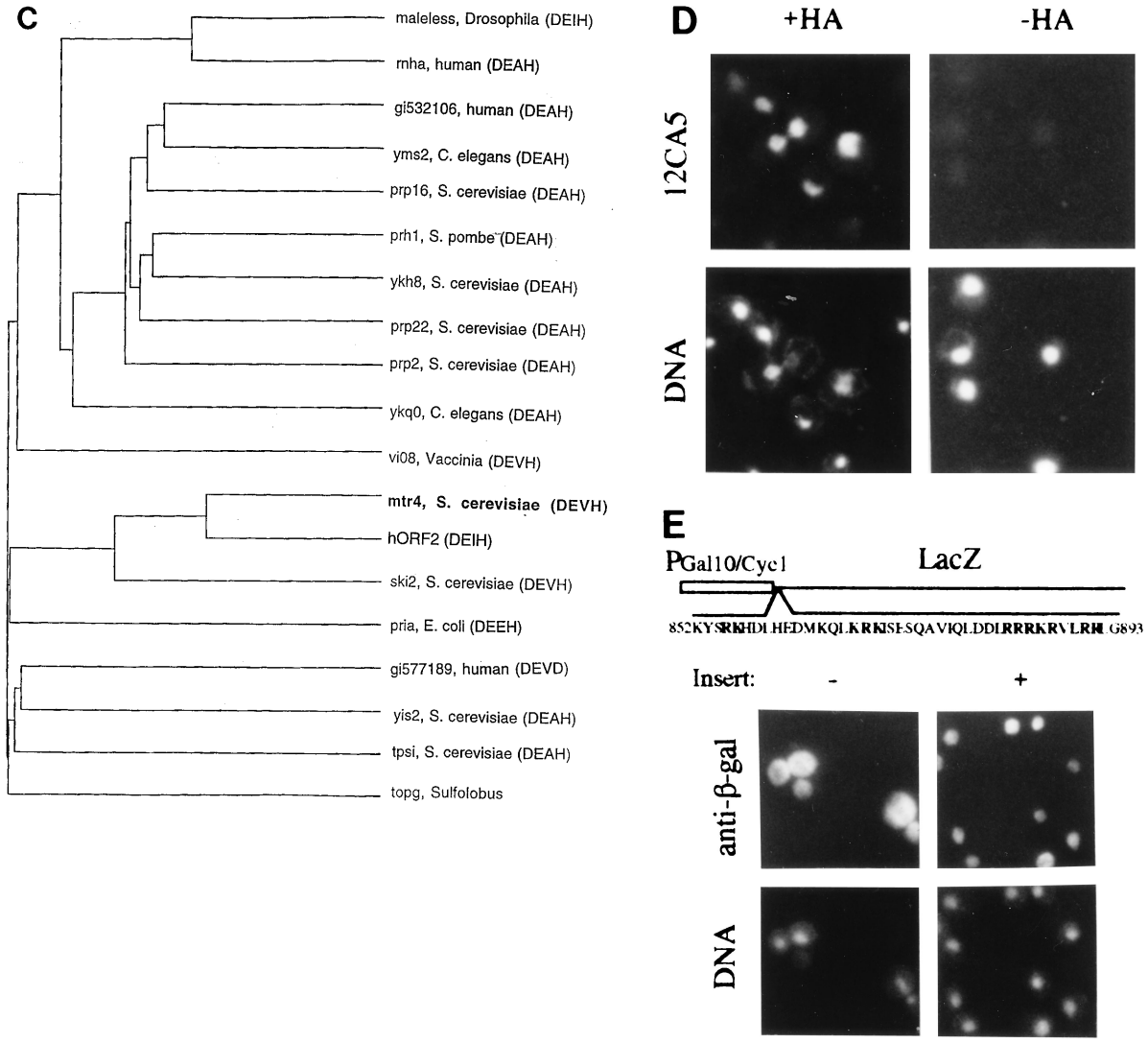


FIG. 1—*Continued.*

RNA by reference to the nucleoporin, Nsp1p, and the nucleolar protein, Nop1p. Poly(A)$^+$ RNA abuts and often overlaps Nsp1p (panel c) and extensively overlaps Nop1p (compare panels b and a). A corresponding accumulation of electron-dense material is seen by electron microscopy (Fig. 2B).

The regions of the nucleus containing the highest concentration of poly(A)$^+$ RNA often appear to deform and protrude from the nuclear envelope. Although in wild-type cells there is no obvious enrichment of Nsp1p or nuclear pores adjacent to the nucleolus (data not shown), light microscopic observations of the Mtr4p depletion strain show that the protruding areas are often enriched in Nsp1p (Fig. 2B, panel c, inset). Moreover, electron microscopic observations show that the electron-dense material (presumably derived from the nucleolus) lies adjacent to portions of the nuclear envelope which have an abundance of nuclear pores (Fig. 2B, panel c).

Mtr4p function appears unique. To learn whether Mtr4p shares functions with other members of the DEAD-box superfamily, we attempted the suppression of known DEAD-box pre-mRNA splicing mutants (the ts *prp2*, *prp5*, *prp16*, *prp22*, and *prp28* strains [30]) and the cold-sensitive *spb4* mutant (32). We also attempted suppression of *mtr4-1* by overexpression of these same DEAD-box members. No rescue was seen at 35 to 37°C (or 16°C for *spb4*). Furthermore, *mtr4-1* was not rescued by overexpression of the GTPase-activating protein *RNA1* (35), the nucleoporins *NSP1* and *NUP1* (8), or *MTR1* or *MTR2.*

Since *SKI2* and human ORF2 cDNA encode proteins related to Mtr4p, we placed these two genes under the control of *ADH1* (alcohol dehydrogenase I) and *GAL1* promoters in a 2μm-based plasmid. Overexpression of Ski2p was tolerated in the wild type but did not rescue growth of *mtr4-1* at 35 or 37°C. Equivalent negative observations were made in attempts to rescue the conditional *GAL10-MTR4*(YSL303) strain carrying *ADH-SKI2* upon shift to glucose medium at 15, 30, 35, or 37°C. Overexpression of human ORF2 in the same vectors reduced cell growth, suggesting that it interacts with critical proteins without fulfilling normal Mtr4p functions.

Biochemistry of Mtr4p. HA-Mtr4p is readily extracted from cells in low salt (i.e., <10 mM NaCl) but is extracted less well in 200 mM NaCl. When protein extracts of cells labelled with Tran^{35}S-label were immunoprecipitated with the anti-HA antibody, HA-tagged Mtr4p was specifically immunoprecipitated,

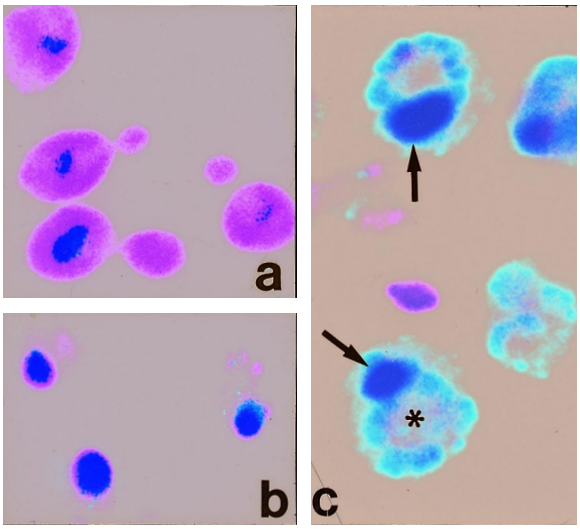
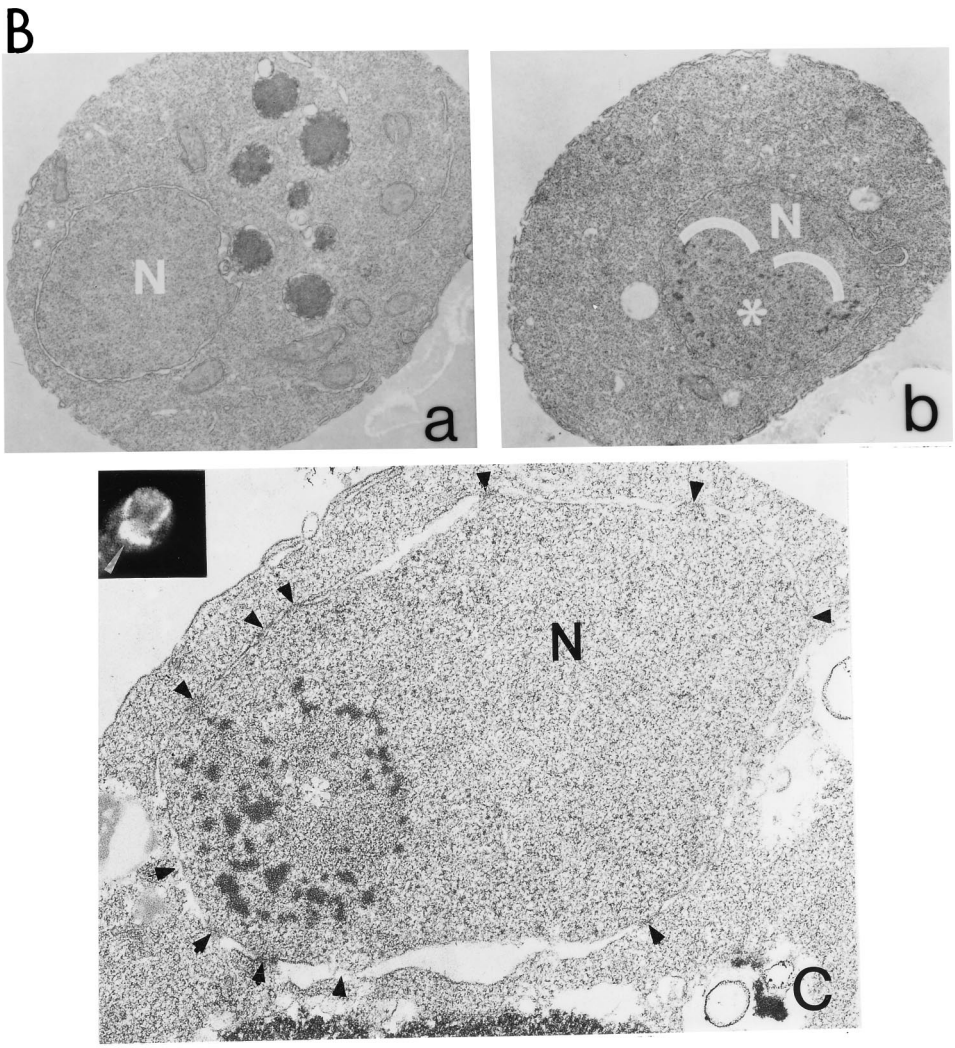


FIG. 2. (A) Localization of poly(A)$^+$ RNA after Mtr4p depletion. Strain YSL303 contains an inducible *MTR4* gene. Cells were examined during growth in medium containing 2% galactose (a) or after a shift to medium containing 2% glucose for 20 h (b and c). Cells were subjected to a double detection of poly(A)$^+$ RNA and the nucleolar antigen Nop1p (a and b) or poly(A)$^+$ RNA and the nuclear pore protein Nsp1p (c). Green represents poly(A)$^+$ RNA. Red represents Nop1p (a and b) or Nsp1p (c). Yellow results from colocalization of poly(A)$^+$ RNA and the antigens. Arrows indicate the accumulated poly(A)$^+$ RNA, which, because of overlap with the Nsp1p signal, is yellow. Note the seemingly empty volume (star) enclosed by the Nsp1p signal. Triple staining shows that the Nop1p-negative region contains the bulk of the nuclear DNA (data not shown). The magnification of panel c is twice that of panels a and b. The cell were examined with a Bio-Rad confocal microscope. (B) Loss of *MTR4* function causes ultrastructural changes in the nucleus. Thin sections of *mtr4-1* (YSL401) at 23°C (a) or after 3 h at 37°C (b) are shown. (c) YSL303 was shifted to glucose medium for 20 h at 30°C. Note the heterogeneous osmiophilic material in the nucleus under restrictive conditions. A normal nucleolus is not seen. N, chromatin-rich nucleoplasm. The stars and arcs designate osmiophilic regions, possibly derived from the nucleolus and including the accumulated poly(A)$^+$ RNA. In panel c, the nuclear pores are designated by arrowheads. Note that, as in the immunofluorescent anti-Nsp1p image in the insert, pores seem to be enriched at the end of the nucleus which is near the osmiophilic material.



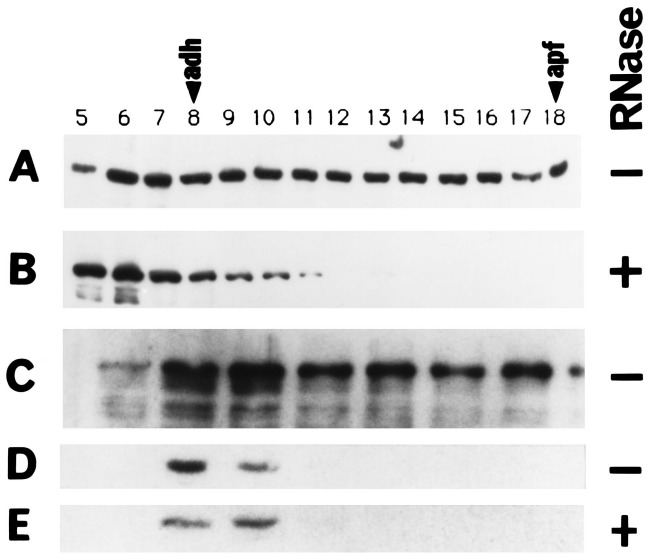


FIG. 3. Glycerol gradient analysis of Mtr4p distribution. (A) Low-salt extracts of cells expressing HA-epitope-tagged Mtr4p were analyzed on 10 to 30% glycerol gradients. Fractions were collected from the top (left) to the bottom (right) and processed by immunoblotting to detect the HA epitope tag. The addition of 2 mM ATP to the extract (or 500 mM KCl) caused only modest changes (data not shown). (B) Digestion with RNase A (10 μg/ml) plus micrococcal nuclease (400 μm/ml) at 30°C for 30 min. (C) Analysis of HA-tagged Mtr4p carrying the *mtr4-1* mutation after depletion of the wild-type protein (as described above) and incubation for 3 h at 37°C. (D to E) Comparable analysis of HA-tagged Mtr2p in YTK206 before (D) and after (E) RNase treatment. Note that the sedimentation of Mtr2p is not altered by RNase. Only fractions 5 to 18 are shown since little signal was seen in fractions 1 to 4 and 19 to 25 or in the pellet. Pairs of fractions were pooled and analyzed for panels C to E. Molecular standards were loaded in parallel gradients: yeast alcohol dehydrogenase (150 kDa) (adh) peaked in fraction 8; apoferritin (443 kDa) (apf) peaked in fraction 18.

but no additional specific bands were detected (data not shown).

Low-salt extracts containing HA-Mtr4p were fractionated on 10 to 30% continuous glycerol gradients and analyzed by Western blotting (immunoblotting). HA-Mtr4p has a broad distribution (Fig. 3A). Treatment with nonspecific RNases before gradient fractionation caused a major change of the distribution of Mtr4p; after RNase treatment, most HA-Mtr4p sediments in fractions 5 to 8, where the monomeric Mtr4p (125 kDa) is expected. Thus, Mtr4p associates, directly or indirectly, with RNA. As shown in panel C, the HA-tagged *mtr4-1* mutant form of Mtr4p was also broadly distributed in comparable experiments. Panels E and F show that HA-Mtr2p (19) does not shift upon RNase treatment.

DISCUSSION

Polydisperse hnRNP particles of higher eukaryotes are thought to be generated from nascent transcripts which are fragmented upon cell disruption (9). Their proteins are largely distinct from those of spliceosomes. Some animal cell hnRNP proteins do shuttle in and out of the nucleus; however, there is no direct demonstation that they convey mRNA to the cytosol (9). By studying *S. cerevisiae*, which has only a few intron-containing genes, we have attempted to focus on the events of transport/export other than those which are related to mRNA splicing. One hnRNP-like protein which appears critical for export of poly(A)$^+$ RNA has been identified (39). The present observation of a putative helicase is consistent with our expectation that normalizing factors may participate in forming or translocating the export RNP itself.

The superfamily of putative DEAD-box helicases includes more than 100 prokaryotic and eukaryotic proteins of diverse function (34). These proteins are customarily described as belonging to the DEAD or DEXH/DEAH subfamilies. Although some show RNA-stimulated ATPase activity, only six are known to melt secondary structure, perhaps because of the need to identify the proper substrates or a need for accessory proteins (15). In addition to their putative roles in mRNA splicing, in which DEAH family members (Prp2p, Prp16p, and Prp22p) appear to catalyze alterations of small nuclear RNA/mRNA interactions (5, 30, 40), these proteins have been implicated in mRNA stabilization (13), mRNA localization (11), and rRNA processing (28, 32). The prototypic DEAD-box protein, eIF-4A, melts the mRNA 5′ secondary structure in the cytosol as part of a complex with p220 and the RNA-binding protein eIF-4B (15). RNA unwinding appears to be the culmination of a sequence of steps: ATP binding, protein conformational change, RNA binding, ATP hydrolysis, stabilization of RNA binding, and finally, unwinding of the RNA.

The identification of structural homologs of Mtr4p is striking. Ski2p functions in the antiviral response, apparently by inhibiting translation of Cap$^-$ poly(A)$^-$ mRNAs in the cytoplasm (23, 44), while hORF2 is known only as a DNA sequence. The extensive similarity of hORF2p to Mtr4p may open the possibility of studying equivalent functions in higher eukaryotes; however, as in many cases in which higher eukaryotic proteins are expressed in yeast cells, this protein cannot replace the Mtr4p function in yeast cells. A further, less closely-related, human homolog—unlike Mtr4p—resides in the nucleolus (7, 21).

We suggest that Mtr4p affects mRNA structure, packaging, or distribution at a late stage of intranuclear transport since, although it is concentrated in the nucleus and binds RNA, it does not appear to be required for pre-mRNA splicing. We will need much more sophisticated means of examining the progress of transcripts within the nucleus, at the biochemical level, before such a suggestion can be critically evaluated. It will also be valuable to identify the set of RNAs to which Mtr4p binds (directly or indirectly) in order to learn whether this protein does have unwinding activity. The observation that the mutant form of Mtr4p still associates with RNA suggests that the carboxy-terminal end of the protein is important for some relatively late step in transport, i.e., subsequent to the initial steps of RNA recognition. In the hope of obtaining some information concerning the role of Mtr4p by relation to other *MTR* products, we generated double mutants between *mtr4-1* and *mtr1-1*, *mtr3-1* and *mtr8-1*, and *mtr12-1* and *mtr13-1*. Each of these parental strains is characterized by a different distribution of poly(A)$^+$ RNA at 37°C (35). No synthetic lethality was observed in any of the resulting double mutants at 23 or 37°C, indicating no strong genetic interaction.

We inquired whether Mtr4p can be immunoprecipitated along with other proteins or whether overexpression of Mtr4p or segments of the protein might be toxic, as might be expected if it interacts with key targets. As mentioned above, no coimmunoprecipitating proteins were detected. With regard to overexpression, an N-terminal fragment of Mtr4p (amino acids 1 to 230) and internally deleted fragments (amino acids 1 to 4 plus 91 to 1073, 1 to 4 plus 334 to 1073, 1 to 229 plus 602 to 1073, 1 to 334 plus 763 to 1073, and 1 to 655 plus 993 to 1073) of HA-tagged Mtr4p were expressed in a wild-type strain (W303) under control of the inducible *GAL10/CYC1* promoter in a low-copy-number vector. Upon induction with 2% galactose, Western blotting confirmed the expression of each spe-

cies. Nevertheless, no toxicity was observed even when the entire Mtr4p was overexpressed (data not shown).

Two other proteins (Mtr13p/Nab1p/Np13p/Nop3p and Nup145p) implicated in RNA export do have complete or partial RNA recognition motifs (4, 8, 31, 39). Possibly related helicase activities have also been detected in the rat liver and in HeLa cell nuclei (10, 21, 36).

The striking observation that depletion of Mtr4p causes accumulation of poly(A)$^+$ RNA in association with the nucleolar antigen, Nop1p, raises exactly the same issues of possible nucleolar involvement in mRNA export which we have recently discussed (35). Three other *mtr* mutants, *mtr3-1*, *mtr14-1*, and *mtr17-1*, share this characteristic with *mtr4-1* (20). A possibly related observation of nucleolar poly(A)$^+$ RNA accumulation has also been made for heat-shocked *Schizosaccharomyces pombe* (41). We favor the idea that the association of mRNA with the nucleolus is the result of interruption of the normal export path under circumstances which still allow RNA translocation and diffusion within the nucleoplasm.

ACKNOWLEDGMENTS

We thank J. Abelson, J. Aris, C. M. Berg, M. Clark, M. Douglas, B. Futcher, L. Guarente, C. Guthrie, A. Hopper, E. Hurt, F. Lacroute, R.-J. Lin, D. McPheeters, N. Nomura, A. Sachs, D. Templeton, and R. Wickner for antibodies, yeast strains and plasmids; T.-H. Chang for the dendrogram; T.-H. Chang, W. Merrick, and J. Woolford for comments on the text; M. Ward for preparing the manuscript, and all members of this laboratory for advice.

This work was supported by NIH grants GM46569 and DK27651 and ACS grant VM-131.

REFERENCES

1. **Amberg, D., A. Goldstein, and C. Cole.** 1992. Isolation and characterization of *RAT1*: and essential gene of *Saccharomyces cerevisiae* required for the efficient nucleocytoplasmic trafficing of mRNA. Genes Dev. **6:**1173–1189.
2. **Berg, C. M., N. Vartak, G. Wang, X. Xu, L. Liu, D. MacNeil, K. Gewain, L. Wiater, and D. Berg.** 1992. The mγδ-1 element, a small γδ (Tn1000) derivative useful for plasmid mutagenesis, allele replacement and DNA sequencing. Gene **113:**9–16.
3. **Bonneaud, N., O. Ozier-Kalogeropoulos, G. Li, M. Labouesse, L. Minvielle-Sebastia, and F. Lacroute.** 1991. A family of low and high copy replicative, integrative and single-strand *S. cerevisiae*/*E. coli* shuttle vectors. Yeast **7:**609–615.
4. **Bossie, M., C. DeHoratius, G. Bartcelo, and P. Silver.** 1992. A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast. Mol. Biol. Cell **3:**875–893.
5. **Chang, T.-H., J. Arenas, and J. Abelson.** 1990. Identification of five putative yeast RNA helicase genes. Proc. Natl. Acad. Sci. USA **87:**1571–1575.
6. **Cheng, Y., J. Dahlberg, and E. Lund.** 1995. Diverse effects of the guanine nucleotide exchange factor RCC1 on RNA transport. Science **267:**1807–1810.
7. **Dangel, A. W., L. Shen, A. R. Mendoza, L.-C. Wu, and C. Y. Yu.** 1995. Human helicase gene *SKI2W* in the HLA class III region exhibits striking structural similarities to the yeast antiviral gene *SKI2* and to the human gene *KIAA0052*: emergence of a new gene family. Nucleic Acids Res. **23:**2120–2126.
8. **Davis, L. I.** 1995. The nuclear pore complex. Annu. Rev. Biochem. **64:**865–896.
9. **Dreyfuss, G., M. J. Matunis, S. Pinol-Roma, and C. G. Burd.** 1993. hnRNP proteins and the biogenesis of mRNA. Annu. Rev. Biochem. **62:**289–321.
10. **Flores-Rozas, H., and J. Hurwitz.** 1993. Characterization of a new RNA helicase from nuclear extracts of HeLa cells which translocates in the 5′ to 3′ direction. J. Biol. Chem. **268:**21372–21383.
11. **Fujiwara, Y., T. Komiya, H. Kawabata, M. Sato, H. Fujioto, M. Furusawa, and T. Noce.** 1994. Isolation of a DEAD-family protein gene that encodes a murine homolog of *Drosophila* vasa and its specific expression in germ cell lineage. Proc. Natl. Acad. Sci. USA **91:**12258–12262.
12. **Guo, Z., and F. Sherman.** 1995. 3′-end-forming signals of yeast mRNA. Mol. Cell. Biol. **15:**5983–5990.
13. **Iost, I., and M. Dreyfus.** 1994. mRNAs can be stabilized by DEAD-box proteins. Nature (London) **372:**193–196.
14. **Izzauralde, E., and I. Mattaj.** 1995. RNA export. Cell **81:**153–160.
15. **Jaramillo, M., T. E. Dever, W. C. Merrick, and N. Sonenberg.** 1991. RNA unwinding in translation: assembly of helicase complex intermediates comprising eukaryotic initiation factors eIF-4F and eIF-4B. Mol. Cell. Biol. **11:**5992–5997.
16. **Jarmolowski, A., W. C. Boelens, E. Izzaurralde, and I. W. Mattaj.** 1994. Nuclear export of different classes of RNA is mediated by specific factors. J. Cell Biol. **124:**627–635.
17. **Jones, E. W.** 1991. Tackling the protease problem in *Saccharomyces cerevisiae*. Methods Enzymol. **194:**428–453.
18. **Kadowaki, T., S. Chen, M. Hitomi, E. Jacobs, C. Kumagai, S. Liang, R. Schneiter, D. Singleton, J. Wisniewska, and A. M. Tartakoff.** 1994. Isolation and characterization of *Saccharomyces cerevisiae m*RNA *t*ransport-defective (*mtr*) mutants. J. Cell Biol. **126:**649–659.
19. **Kadowaki, T., M. Hitomi, S. Chen, and A. M. Tartakoff.** 1994. Nuclear mRNA accumulation causes nucleolar fragmentation in yeast *mtr2* mutant. Mol. Biol. Cell **5:**1253–1263.
20. **Kadowaki, T., R. Schneiter, M. Hitomi, and A. M. Tartakoff.** 1995. Mutations in nucleolar proteins lead to nucleolar accumulation of polyA+RNA in *Saccharomyces cerevisiae*. Mol. Biol. Cell **6:**1103–1110.
21. **Lee, G.-C., and J. Hurwitz.** 1992. A new helicase isolated from HeLa cells that catalyticaly translocates in the 3′ to 5′ direction. J. Biol. Chem. **267:**4398–4407.
22. **Lee, S.-G., I. Lee, S. H. Park, C. Kang, and K. Song.** 1995. Identification and characterization of a human cDNA homologous to yeast SKI2. Genomics **25:**660–666.
23. **Masison, D., A. Blanc, J. C. Ribas, K. Carroll, N. Sonenberg, and R. B. Wickner.** 1995. Decoying the Cap$^-$ mRNA degradation system by a double-stranded RNA virus and poly(A)$^-$ mRNA surveillance by a yeast antiviral system. Mol. Cell. Biol. **15:**2763–2771.
24. **Munroe, S. H., and X. Dong.** 1992. Heterogeneous nuclear ribonucleoprotein A1 catalyzes RNA-RNA annealing. Proc. Natl. Acad. Sci. USA **89:**895–899.
24a. **Nomura, N., et al.** Unpublished data.
25. **Oberosler, P., P. Hloch, U. Ramsperger, and H. Stahl.** 1993. p53-catalyzed annealing of complementary single-stranded nucleic acids. EMBO J. **12:**2389–2396.
26. **Pause, A., N. Méthot, and N. Sonenberg.** 1993. The HRIGRXXR region of the DEAD box RNA helicase eukaryotic translation initiation factor 4A is required for RNA binding and ATP hydrolysis. Mol. Cell. Biol. **13:**6789–6798.
27. **Plumpton, M., M. McGarvey, and J. D. Beggs.** 1994. A dominant negative mutation in the conserved RNA helicase motif SAT causes splicing factor PRP2 to stall in spliceosomes. EMBO J. **13:**879–887.
28. **Ripmaster, T. L., G. P. Vaughn, and J. L. Woolford, Jr.** 1992. A putative ATP-dependent RNA helicase involved in *Saccharomyces cerevisiae* ribosome assembly. Proc. Natl. Acad. Sci. USA **89:**11131–11135.
29. **Rose, M. D., and J. R. Broach.** 1991. Cloning genes by complementation in yeast. Methods Enzymol. **194:**195–230.
30. **Ruby, S. W., and J. Abelson.** 1991. Pre-mRNA splicing in yeast. Trends Genet. **7:**79–85.
31. **Russell, I., and D. Tollervey.** 1995. Yeast Nop3p has structural and functional similarities to mammalian pre-mRNA binding proteins. Eur. J. Cell Biol. **66:**293–301.
32. **Sachs, A. B., and R. W. Davis.** 1990. Translation initiation and ribosomal biogenesis: involvement of a putative rRNA helicase and RPL46. Science **247:**1077–1079.
33. **Schiestl, R., and R. Gietz.** 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as carrier. Curr. Genet. **16:**339–346.
34. **Schmid, S. R., and P. Linder.** 1992. D-E-A-D protein family of putative RNA helicases. Mol. Microbiol. **6:**283–292.
34a. **Schneiter, R., et al.** Unpublished data.
35. **Schneiter, R., T. Kadowaki, and A. M. Tartakoff.** 1995. mRNA transport in yeast: time to reinvestigate the functions of the nucleolus. Mol. Biol. Cell **6:**357–370.
36. **Schroder, H. C., D. Ugarkovic, P. Langen, M. Bachmann, A. Dorn, Y. Kuchino, and W. E. G. Muller.** 1990. Evidence for involvement of a nuclear envelope-associated RNA helicase activity in nucleocytoplasmic RNA transport. J. Cell. Physiol. **145:**136–146.
37. **Shuman, S.** 1992. Vaccinia virus RNA helicase: an essential enzyme related to the DE-H family of RNA-dependent NTPases. Proc. Natl. Acad. Sci. USA **89:**10935–10939.
38. **Sikorski, R., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
39. **Singleton, D. R., S. Chen, M. Hitomi, C. Kumagai, and A. M. Tartakoff.** 1995. A yeast protein that bidirectionally affects nucleocytoplasmic transport. J. Cell Sci. **108:**265–272.
40. **Strauss, E. J., and C. Guthrie.** 1991. A cold-sensitive mRNA splicing mutant is a member of the RNA helicase gene family. Genes Dev. **5:**629–641.
41. **Tani, T., R. Derby, Y. Hiraoka, and D. Spector.** 1995. Nucleolar accumulation of poly(A)+ RNA in heat-shocked yeast cells: implication of nucleolar involvement in mRNA transport. Mol. Biol. Cell **6:**1515–1534.
42. **Visa, N., A. Alzhanova-Ericsson, X. Sun, E. Kiseleva, B. Bjorkroth, T. Wurtz, and B. Daneholt.** 1996. A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell **84:**253–264.
43. **Wahle, E., and W. Keller.** 1992. The biochemistry of 3′-end cleavage and polyadenylation of messenger RNA precursors. Annu. Rev. Biochem. **61:**419–440.
44. **Widner, W. R., and R. B. Wickner.** 1993. Evidence that the *SKI* antiviral system of *Saccharomyces cerevisiae* acts by blocking expression of viral mRNA. Mol. Cell. Biol. **13:**4331–4341.