MOLECULAR AND CELLULAR BIOLOGY, Sept. 1988, p. 3636–3646
0270-7306/88/093636-11$02.00/0

Vol. 8, No. 9

Structure and Regulation of a Nuclear Gene in *Saccharomyces cerevisiae* That Specifies MRP7, a Protein of the Large Subunit of the Mitochondrial Ribosome

KATHLEEN FEARON AND THOMAS L. MASON*

Department of Biochemistry and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, Massachusetts 01003

Received 8 February 1988/Accepted 17 May 1988

The gene for MRP7, a 40-kilodalton protein of the large subunit of the yeast mitochondrial ribosome, was identified in a λgt11 expression library by immunological screening with a monoclonal antibody to MRP7. An intact copy of *MRP7* was then isolated from a yeast genomic library by colony hybridization. Gene disruption showed that MRP7 protein was essential for ribosomal function. Sequencing of *MRP7* revealed a coding region for a basic (pI 10.6), 43.2-kilodalton protein containing 371 amino acid residues. Amino acid residues 28 to 112 of the deduced MRP7 sequence aligned with the 84 residues of the *Escherichia coli* ribosomal protein L27, but no significant similarity was detected between the carboxy-terminal 259 amino acids of MRP7 and other protein sequences in existing computer data bases. Within the aligned region, there was 49% amino acid identity between MRP7 and L27, compared with the 57% identity observed between L27 and its homolog in *Bacillus stearothermophilus*. The steady-state levels of the MRP7 protein and its mRNA were monitored in response to catabolite repression and to increased dosage of the *MRP7* gene. The response to catabolite repression was characterized by a ninefold change in the level of the protein and little, if any, change in the level of the mRNA. In cells carrying the *MRP7* gene on a high-copy-number plasmid, the mRNA was increased 20-fold, but there was no significant increase in MRP7 protein. Furthermore, *MRP7* mRNA and protein accumulated at normal levels in [*rho*^{0}] cells, which are devoid of 21S rRNA, indicating that the protein is relatively stable in the absence of ribosome assembly. Together, these results suggest that *MRP7* is regulated posttranscriptionally, probably at the level of protein synthesis rather than protein turnover.

Ribosomes are universal features of procaryotic and eucaryotic cells, and intensive investigation for over two decades has led to major advances in our understanding of their structure, function, synthesis, and evolution (for reviews, see references 16, 28, 31, 40, 44, and 66). Much of this knowledge has been developed in studies of the *Escherichia coli* ribosome, which has become the standard of comparison for the analysis of ribosomes in other organisms. In contrast to bacteria, eucaryotic cells maintain multiple, distinctly different ribosomal populations for protein synthesis in the cytoplasm, chloroplasts, and mitochondria. The presence of cytoplasmic and organellar ribosomes provides an opportunity to compare the properties of genetically separate sets of ribosomal components within a single cell and to establish relationships between these components and those of the *E. coli* ribosome. These comparisons should help to identify features of eucaryotic ribosomes that are special adaptations to the complex genomic organization and intracellular compartmentalization of eucaryotic cells. The ribosomes of mitochondria and chloroplasts are of special interest in this regard because the genes for the constituents of organellar ribosomes are divided between two genetic systems (6, 65).

In the case of the mitochondrial ribosome of *Saccharomyces cerevisiae*, the 15S and 21S rRNAs and one ribosomal protein are encoded by mitochondrial DNA (mtDNA) (65). The remaining 60 to 70 ribosomal proteins are specified by nuclear genes, translated on cytoplasmic ribosomes, and imported into the mitochondria. The ribosomal genes in mtDNA have been identified and sequenced, but little is known about the genes for the nucleus-encoded ribosomal proteins.

Recently, two nuclear genes for yeast mitochondrial ribosomal proteins were isolated through the analysis of *pet* mutants with impaired mitochondrial protein synthesis (39). *MRP1* (for mitochondrial ribosomal protein) codes for a basic 37-kilodalton (kDa) polypeptide with no significant relatedness to any other ribosomal protein for which sequence information is available. *MRP2* specifies a 14-kDa polypeptide that is related to the *E. coli* ribosomal protein S14 and to a chloroplast-encoded protein of chloroplast ribosomes. Although the original *pet* alleles of *MRP1* and *MRP2* have leaky phenotypes, null mutants created by gene disruption are devoid of mitochondrial protein synthesis, showing that the two proteins are essential for ribosomal function. The ability to identify an *E. coli* homolog for MRP2 but not MRP1 suggests that some mitochondrial ribosomal proteins have changed enough through evolution to preclude the easy identification of their relationship to other ribosomal proteins through sequence comparisons. Alternatively, a protein such as MRP1 may have arisen from a nonribosomal ancestor, which was recruited during evolution to provide an ancillary function unique to mitochondrial protein synthesis.

With a view to developing a broad perspective of the functional and evolutionary properties of mitochondrial ribosomal proteins in *S. cerevisiae*, we have identified several nuclear genes for components of the mitochondrial ribosome through immunological screening of λgt11 genomic expression libraries. In this paper we describe the isolation and characterization of *MRP7*, the gene for a 40-kDa protein of the large subunit. The data demonstrate that a short amino-

* Corresponding author.

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
MH41-7Bρ$^+$	a *ade2 his1* [*rho*$^+$]	M. Bolotin-Fukahara
MH41-7Bρ^0	a *ade2 his1* [*rho*^{0}]	M. Bolotin-Fukahara
YNN281	a *trp1 his3 lys2-801a ade2 ura3-52*	YGSC[a]
YNN282	α *trp1 his3 lys2-801a ade2 ura3-52*	YGSC
KFY1	α *trp1 his3 lys2-801a ade2 mrp7::URA3* [*rho*$^-$]	This study
KFY3	α *trp1 his3 lys2-801a ade2 mrp7Δ::URA3* [*rho*$^-$]	This study
KFY6	a/α *trp1/+ his3/+ lys2-801a/+ ade2/ade2 his1/+ MRP7/mrp7::URA3* [*rho*$^-$]	This study
SB9882-4CR	a/α *ura3-52/ura3-52 trp1-289/trp1-289 leu2-3,112/+ his4-519/+ can1 cry*^{r}	J. Carbon
KFY2	a/α *trp1-289/trp1-289 leu2-3,112/+ his4-519/+ can1 cry*^{r} *ura3-52/mrp7::URA3* [*rho*$^+$]	This study
KFY13	α *mrp7::URA3 leu2-3,112 his4-519 trp1-289* [*rho*$^-$]	This study
22-2D	α *ura3-52 trp1 leu2-3,112 cyh2 can1*	G. R. Fink
KFY41	α *ura3-52 trp1 leu2-3,112 cyh2 can1* pKF33 (2μm, *URA3*, *MRP7*)	This study
KFY43	α *ura3-52 trp1 leu2-3,112 cyh2 can1* pKF27 (2μm, *URA3*, *GAL1-MRP7*)	This study
M7-40	a *ade1* [*mit*$^-$ (*cob1*)]	54
M9-94/4B	a *ade1* [*mit*$^-$ (*oxi1*)]	54
M9-3/5C	a *ade1* [*mit*$^-$ (*oxi2*)]	54
M10-150/4D	a *ade1* [*mit*$^-$ (*oxi3*)]	54
KL14-4A-30A	a *his1 trp2* [*rho*$^-$ (*cob* region)]	54

[a] YGSC, Yeast Genetic Stock Center.

terminal domain in MRP7 contains a remarkably conserved copy of the much smaller (9-kDa) *E. coli* ribosomal protein L27. In addition, we show that expression of *MRP7* is regulated posttranscriptionally, probably at the level of protein synthesis rather than degradation.

MATERIALS AND METHODS

Immunological screening of phage plaques. We have isolated a hybridoma cell line (D. J. Perry and T. L. Mason, unpublished) that secretes a monoclonal antibody to a 40-kDa protein which cosediments with the 50S subunit of the mitochondrial ribosome in high-salt sucrose gradients. This protein is the seventh largest of the total mitochondrial ribosomal proteins, as estimated by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (SDS) (14), and has been designated MRP7. Hybridoma cell culture supernatant was used to immunologically screen a λgt11 yeast genomic library (72, 73). Standard methods for phage growth, storage, and DNA preparation were used (55).

Yeast strains and growth media. The yeast strains used in this study are described in Table 1. All yeast transformations were performed by the lithium acetate procedure described by Ito et al. (22). One-step gene transplacement was used to disrupt the genomic copy of *MRP7* in both haploid and diploid strains (48). Strains KFY1 and KFY2 were obtained by transforming YNN282 and SB9882-4CR, respectively, with the *Eco*RI fragment shown in Fig. 1B. KFY3 was obtained by transforming YNN282 with the *Eco*RI fragment shown in Fig. 1C. KFY41 and KFY43 were obtained by transforming strain 22-2D with the multicopy plasmids pKF33 and pKF27, respectively (plasmids are described in Fig. 3). Sporulation and tetrad dissection of KFY2 gave rise to KFY13. Cells were grown in rich (1% yeast extract, 2% peptone) or minimal (0.67% yeast nitrogen base [Difco] without amino acids) medium. The minimal medium was also supplemented with amino acids, minus either leucine or uracil, to select for the appropriate plasmid. All media were supplemented with either 5% glucose, 2% galactose, or 2% glycerol plus 2% ethanol as carbon sources.

KFY13 was crossed to M7-40, M9-94/4B, M9-3/5C, M10-150/4D, KL14-4A-30A, and MH41-7B [*rho*$^-$] (Table 1), and diploids were scored for their ability to grow on rich medium supplemented with 2% glycerol plus 2% ethanol as the sole carbon source.

DNA manipulations. Standard procedures were used for *E. coli* transformation and in vitro manipulation of DNA (34). Enzymes were used according to the specifications given by the supplier. Plasmids used in this study were constructed as follows. For pKF1, the 1.4-kilobase (kb) *Eco*RI fragment from λ*MRP7* (Fig. 1) was ligated into the unique *Eco*RI site of pACYC184 (3). For pKF3 and pKF4, the *URA3* gene flanked by *Xba*I sites was excised from pRa545 (kindly provided by Kelly Tatchell [61]) and inserted into the *MRP7* coding region after partial *Xba*I digestion of pKF1. The resulting plasmids, pKF3 (insertion) and pKF4 (deletion), were the source of DNA fragments for replacing the genomic copy of *MRP7* by integrative transformation. The plasmid pKF5 (see Fig. 3A for restriction map) was isolated by colony hybridization (17) of a yeast genomic library in YCp50 (47). Religation of *Bgl*II- and *Eco*RI-digested pKF5 produced the subclone pKF11, which contains the 2.9-kb insert shown in Fig. 3B and a 2.6-kb *Bgl*II-*Eco*RI fragment from YCp50 containing the *E. coli* origin of replication and the gene for ampicillin resistance. Plasmids pKF15 to pKF23 were generated by partially digesting pKF11 with *Hin*dIII and gel-purifying the linear DNA fragments between 4.1 and 5.5 kb. The *Hin*dIII sites were filled in, and nonphosphorylated *Bam*HI linkers [d(CGGGATCCCG)] were ligated to the fragments (52), generating a series of linker insertions and deletions within and flanking the *MRP7* coding region. pKF15 contains a *Bam*HI linker at the 5′-most *Hin*dIII site of pKF11 (see Fig. 3B). pKF20 contains a *Bam*HI linker at the 3′-most *Hin*dIII site of pKF11. To obtain pKF24 a *Bam*HI linker was inserted at the unique *Eco*RI site of pKF15 (described above). For pKF27, the 1.7-kb *Bam*HI fragment from pKF24 containing the *MRP7* coding region was inserted at the unique *Bam*HI site of pB656 (provided by G. R. Fink) in an orientation so that *MRP7* was under the

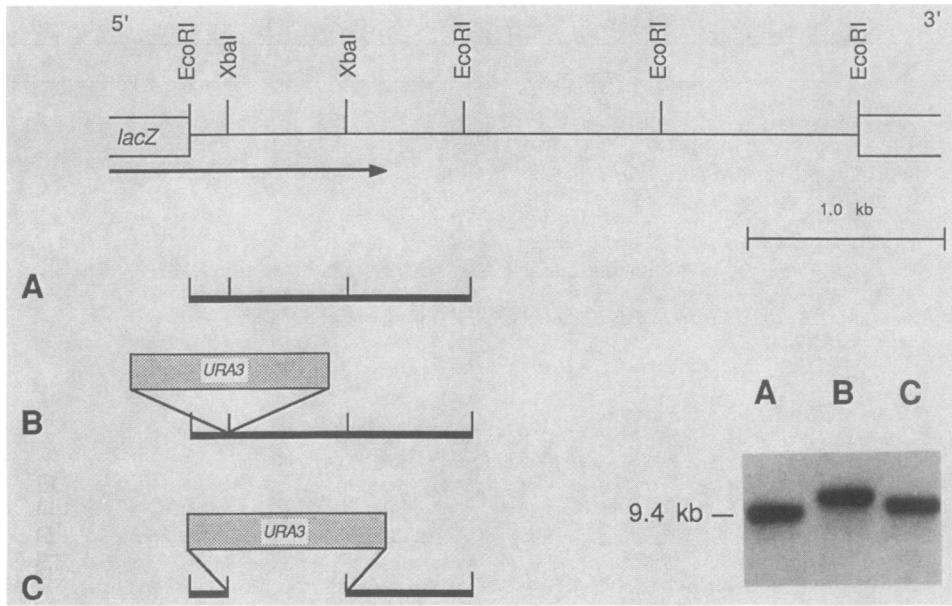

FIG. 1. Restriction map of λ*MRP7* and structure of the mutations generated in *MRP7*. The restriction map of λ*MRP7* is shown at the top. The arrow indicates the direction of translation from *lacZ*. The two internal *Eco*RI sites are genomic sites, and the *Eco*RI sites flanking the 3.4-kb insert are from *Eco*RI linkers added during construction of the expression library. Below the λ*MRP7* map are the fragments used to disrupt *MRP7*. (A) The 1.4-kb *Eco*RI fragment that was subcloned back into λgt11 and into pACYC184, creating λ*MRP7-1* and pKF1, respectively (for details, see Materials and Methods). (B) pKF3 was obtained by insertion of a 1.1-kb *Xba*I fragment containing the *URA3* gene into the upstream *Xba*I site of *MRP7*. (C) pKF4 was generated by replacing the 600-bp *Xba*I fragment with *URA3*. The *Eco*RI fragments shown in panels B and C were used to disrupt the genomic copy of *MRP7* in the haploid strain YNN282. The autoradiograph on the right is a Southern blot of *Eco*RI-digested genomic DNA from yeast strains (A) wild-type YNN282, (B) KFY1 (*mrp7*::*URA3*), and (C) KFY3 (*mrp7*Δ::*URA3*). The Southern blot was probed with the nick-translated 1.4-kb *Eco*RI fragment from λ*MRP7*.

control of the *GAL1* promoter (see Fig. 3C). The plasmid pB656 contains 795 base pairs (bp) of the *GAL1* promoter, including the UAS and transcriptional start site, to within 4 nucleotides of the *GAL1* translational start site (5). pB656 also contains the *E. coli* origin of replication, the gene for ampicillin resistance, yeast 2μm sequences, and the yeast *URA3* gene. For pKF33, the 2.6-kb *Bgl*II-*Bam*HI fragment from pKF20 was inserted at the *Bam*HI site of YEp13 (see Fig. 3D).

DNA sequence analysis. The DNA sequence was determined by the dideoxy chain termination method (50, 51) with M13 clones (36). The clones were constructed by using naturally occurring restriction sites, and nested deletions were generated by the method of Dale et al. (9). Both strands were sequenced. The 371-amino-acid open reading frame of *MRP7* was compared with sequences in the National Biomedical Research Foundation (NBRF) protein data base by the method of Wilbur and Lipman (69).

S1 nuclease protection. S1 nuclease experiments were carried out essentially as described by Favaloro et al. (13). The 2.6-kb *Xba*I fragment and the 3.6-kb *Xho*I fragment from pKF5 were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The *Xba*I and *Xho*I fragments were digested again with *Bgl*II and *Xba*I, respectively. The appropriate fragments were gel-purified, and 50,000 cpm was combined with 50 μg of total RNA from yeast strain MH41-7B [*rho*$^+$] grown in rich medium supplemented with 2% glycerol and 2% ethanol. After denaturing at 75°C for 5 min, hybridization was continued at 42°C for 16 to 20 h in 70% formamide–0.1 M PIPES [piperazine-*N*,*N*′-bis[2-ethanesulfonic acid), pH 6.4]–0.01 M EDTA–0.4 M NaCl. S1 digestion was done at 37°C for 30 min with different concentrations of enzyme, ranging from 50 to 400 U. Samples were separated on a 6% polyacrylamide sequencing gel, with a sequencing reaction as a size standard.

Southern blot analysis. Southern blot analysis was performed essentially as described by Southern (57). Total yeast DNA was isolated as described (54).

Northern (RNA) blot analysis. Total yeast RNA was isolated as described previously (58), and the concentration of RNA was determined by measuring the A_{260}. Equal amounts of total RNA were loaded in each lane of a 1% agarose–6% formaldehyde gel with 0.1 M $NaPO_4$, pH 7.0, as a buffer. The RNA was transferred to a nylon membrane (0.45-μm pore size; Schleicher & Schuell) and probed with the appropriate nick-translated fragment by the method of Thomas (62).

Western (immunoblot) analysis. Two methods were used to prepare extracts of total yeast proteins: (i) alkaline hydrolysis and trichloroacetic acid (TCA) precipitation (71) or (ii) breakage of whole cells with glass beads and solubilization in 2.5% SDS (10). Mitochondrial fractions were prepared as described elsewhere (10). Proteins were resolved on 12.5% polyacrylamide gels in the presence of SDS (27) and electrophoretically transferred to nitrocellulose (0.45 μm) by the method of Towbin et al. (63). The blot was treated in one of two ways: (i) saturated in incubation buffer (20 mM Tris hydrochloride [Tris-HCl, pH 7.4], 0.9% NaCl, 1% bovine serum albumin, 0.01% NaN_3), incubated with the appropriate antibody in incubation buffer, and washed three times in wash buffer (20 mM Tris-HCl [pH 7.4], 0.9% NaCl, 0.1% bovine serum albumin, 0.05% Nonidet P-40, 0.01% NaN_3), or (ii) saturated with 5% nonfat dry milk, as described by Johnson et al. (23). As the second antibody, we used ^{125}I-labeled rabbit anti-mouse immunoglobulins, prepared by the method of Hunter and Greenwood (20).

Quantitation of RNA and protein. Protein concentration

was determined by the method of Lowry et al. (32), and mRNA concentration was determined by measuring the A_{260} (1.0 A_{260} unit = 40 μg). To confirm that equal amounts of protein were loaded in each lane of a gel, samples were run in duplicate and one-half of the gel was stained with bromophenol blue. The levels of mRNA and protein on Northern and Western blots, respectively, were estimated by scanning densitometry of the autoradiograms (Kodak SB-5 X-ray film) with a Hoefer Scientific GS300 scanning densitometer.

RESULTS

Identification of the *MRP7* coding region. A monoclonal antibody to MRP7, a 40-kDa large-subunit protein from the yeast mitochondrial ribosome, was used to identify *MRP7*-specific recombinants in a yeast genomic library constructed in the phage expression vector λgt11 (72, 73). Three immunopositive recombinants were obtained from a screen of 500,000 phage. Restriction mapping of the phage DNA showed that all three recombinants contained the same 3.4-kb yeast DNA insert. One of these clones, designated λ*MRP7*, was chosen for further analysis. The restriction map of the insert in λ*MRP7* is shown in Fig. 1.

The next step in our analysis was to gain information about the position and orientation of the sequences encoding the MRP7 antigen in λ*MRP7*. We first tried to identify a β-galactosidase-MRP7 fusion protein in stained gels and on Western blots of protein extracted from λ*MRP7*-infected, isopropyl-β-D-thiogalactopyranoside (IPTG)-induced *E. coli* cells. Although a 117-kDa *lacZ* polypeptide was clearly identifiable by staining with Coomassie blue and by reactivity with a monoclonal antibody to *E. coli* β-galactosidase, there was no indication of a higher-molecular-weight fusion protein or any other polypeptide with reactivity to the monoclonal antibody against MRP7 (data not shown). These results suggested two possibilities: either the MRP7 antigen was not stable as a fusion protein in *E. coli*, or it was expressed independently of *lacZ*, as has been demonstrated in other cases (56). Further tests were performed to help locate the *MRP7* sequences within the λ*MRP7* insert. First, the expression of the MRP7 antigen from λ*MRP7* was shown to be IPTG inducible in a plaque-lift assay and thus *lacZ* dependent as defined by Snyder et al. (56). Second, the 1.4-kb *Eco*RI fragment at the 5′ end (with respect to *lacZ*) of the yeast insert in λ*MRP7* was subcloned back into λgt11 in both orientations. Only the recombinants with the fragment inserted in the original orientation produced immunopositive plaques. Finally, nucleotide sequencing of the 5′ end of the 1.4-kb *Eco*RI fragment from λ*MRP7* showed that an open reading frame of at least 75 codons was fused in-frame with *lacZ*. Together, these results supported the assumption that this open reading frame was within the MRP7 protein-coding region, and we proceeded on the basis of that assumption.

To confirm that we had in fact identified DNA sequences corresponding to the gene for the MRP7 protein, disruptions were created by inserting a 1.1-kb *Xba*I fragment containing the yeast *URA3* gene within the predicted MRP7 coding region as shown in Fig. 1. The linear fragments carrying the *URA3* inserts were excised from the respective plasmids by digestion with *Eco*RI and used to replace genomic sequences by integrative transformation (48). Ura$^+$ transformants of both the haploid strain YNN282 and the diploid strain SB9882-4CR were isolated and checked for their ability to grow on nonfermentable carbon sources (glycerol plus ethanol). As expected for the inactivation of a *PET* gene, the haploid Ura$^+$ transformants were respiration deficient and the diploid transformants were respiration competent. Southern blot analysis of the genomic DNA from representative haploid transformants verified that, in each case, integration had been directed to the putative *MRP7* locus (Fig. 1). These results corroborated earlier Southern analyses (not shown), which demonstrated that the cloned DNA was present in a single copy in the yeast genome. In each of over 20 tetrads analyzed from sporulated diploid transformants, respiration deficiency and Ura$^+$ cosegregated 2:2, providing further evidence that the two phenotypes were the result of the same genetic event.

The inactivation of genes whose functions are required for mitochondrial protein synthesis has been shown to lead not only to respiratory deficiency but also to instability of the mitochondrial genome and rapid, quantitative conversion to a mixed population of [*rho*^{0}] and [*rho*$^-$] petites (38, 39). Therefore, if mitochondrial protein synthesis is indeed necessary for the maintenance of [*rho*$^+$] mitochondrial DNA and *MRP7* provides an essential function in the mitochondrial ribosome, then the haploid Ura$^+$ transformants generated above should be *mrp7*::*URA3* [*rho*$^-$] double mutants. This possibility was tested genetically by crossing *mrp7*::*URA3* mutants to *MRP7* tester strains with [*rho*^{0}], [*rho*$^-$], and [*mit*$^-$] mtDNA (see Materials and Methods) and checking for the restoration of respiration in the resulting diploids. The diploids issued from crosses to the [*rho*^{0}] and [*rho*$^-$] test strains were uniformly respiration deficient. Some respiration-competent diploids were obtained from the crosses to the [*mit*$^-$] testers. These results show that the *mrp7*::*URA3* parents tested were all either [*rho*^{0}] or [*rho*$^-$]. That some of the mutants were [*rho*$^-$], not [*rho*^{0}], was indicated by the retention of mtDNA capable of complementing [*mit*$^-$] mutations. Thus, the *mrp7*::*URA3* strains were all cytoplasmic petites, providing independent support to the hypothesis that mitochondrial protein synthesis is necessary for the maintenance of wild-type mtDNA (38).

Verification that the expression of the MRP7 protein was affected in the *mrp7*::*URA3* strains was obtained from immunoblot analyses of proteins extracted from the mutants (Fig. 2). Monoclonal antibodies to MRP7 and to subunit II of cytochrome *c* oxidase (coxII) were used to monitor levels of both MRP7 and a representative mitochondrial translation product. As expected, neither protein was detectable in extracts from the *mrp7*::*URA3* mutant. However, both proteins were present in the *MRP7*/*mrp7*::*URA3* heterozygous diploid. To show that the loss of MRP7 was not a secondary effect of the conversion to [*rho*$^-$], an *MRP7*/*mrp7*::*URA3* [*rho*$^-$] diploid was formed by mating KFY1 (α *mrp7*::*URA3* [*rho*$^-$]) to MH41-7B (**a** *MRP7* [*rho*^{0}]). The MRP7 protein was present in the resulting diploid KFY6 but coxII was not, clearly showing that *MRP7* is expressed normally in [*rho*$^-$] cells (compare lanes 3 and 4, Fig. 2). These results strongly support the previous assumption that part of the *MRP7* gene is present at the 5′ end of the yeast DNA insert in λ*MRP7*.

The next step was to isolate the intact *MRP7* gene. The 1.4-kb *Eco*RI fragment from λ*MRP7* was used as a probe in colony hybridization to identify *MRP7*-containing plasmids in a YCp50 yeast genomic library (provided by Mark Rose [47]). Three plasmids (out of 4,000 colonies screened) were isolated and characterized by restriction mapping. From a comparison with the map of the insert in λ*MRP7* (Fig. 3), it seemed likely that the 12-kb insert in pKF5 contained a functional copy of *MRP7*, and this plasmid was chosen for further analysis. Because conversion to [*rho*$^-$] is irreversible, it was not possible to test directly for *MRP7* function by introducing pKF5 into KFY13 (α *mrp7*::*URA3 leu2-3,112*

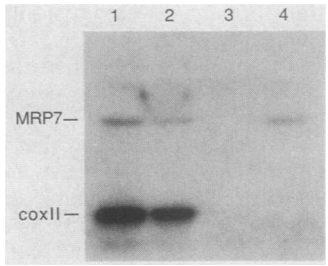

FIG. 2. Immunoblot analysis of total cell proteins from wild-type and *MRP7* mutant yeast strains. Cells were harvested after growth to mid-exponential phase in YPD (1% glucose), and mitochondrial lysates were prepared by breakage with glass beads and solubilization of the mitochondrial fraction with SDS. A 100-μg amount of total cell protein was resolved on a 12.5% polyacrylamide gel. Proteins were transferred to nitrocellulose, and the filter was saturated with incubation buffer. Following incubation with monoclonal antibodies to MRP7 and coxII, a mitochondrial translation product, the immune complexes were decorated with ^{125}I-labeled second antibody. Lane 1, YNN282, the wild-type haploid; lane 2, KFY2, **a**/α *MRP7/mrp7::URA3* [*rho*$^+$]; lane 3, KFY1, α *mrp7::URA3*; lane 4, KFY6, **a**/α *MRP7/mrp7::URA3* [*rho*$^-$], derived by crossing KFY1 to MH41-7B [*rho*^{0}]. The faint band at the position of coxII in lane 3 is due to cross-contamination from the adjacent lane and was not seen in other Western blots of the same protein sample.

his4-519 trp1-289 [*rho*$^-$]) and selecting for respiration-competent transformants. Rather, we first introduced pKF5 (*URA3*) into the wild-type strain YNN281 (**a** *MRP7 ura3-52 Δtrp1 his3Δ200 lys2-801a ade2-1*$_0$ [*rho*$^+$]), and a Ura$^+$ transformant was then mated to KFY13. Tetrads from the sporulated diploid were dissected and analyzed for respiration competence and Ura$^+$. Since *mrp7::URA3* and the centromere-containing pKF5 segregated independently in meiosis, it was possible to identify tetrads in which all four spores grew on nonfermentable carbon sources and Ura$^+$ segregated 2:2. Southern blot analysis of *Bgl*II-digested DNA from one of these tetrads (data not shown) confirmed that the *mrp7::URA3* allele and pKF5 were both present in the Ura$^+$ spores. We thus concluded that pKF5 contains a functional gene for the MRP7 protein.

Sequence of *MRP7*. The strategy used to sequence *MRP7* is shown at the bottom of Fig. 3. Approximately 2.0 kb of the 2.9-kb *Bgl*II-*Eco*RI fragment were sequenced in both directions by the chain termination method (51). Subclones for sequencing were derived from λ*MRP7* and pKF5 by using convenient restriction sites and by generating nested deletions by the method of Dale et al. (9). The nucleotide sequence and the predicted amino acid sequence of *MRP7* are shown in Fig. 4. The sequence reveals a single long open reading frame of 1,113 nucleotides, which could encode a 371-amino-acid protein with a pI of 10.6 and a calculated M_r of 43,223. This corresponds well with the estimated size (40 kDa) of the mature polypeptide, assuming that MRP7 is synthesized in the form of a precursor molecule with a cleavable mitochondrial leader sequence. The amino-terminal sequence of 27 amino acids had a net positive charge of +2 and contained three serines, properties which are consistent with those of known mitochondrial leader peptides (11, 21).

The pattern of codon usage in the *MRP7* coding region is characteristic of lowly expressed yeast proteins (53). The AUG of the *MRP7* open reading frame was preceded by an A at −3, which has been proposed to be important for efficient translation initiation in eucaryotes (26).

A comparison of the amino acid sequence of MRP7 with

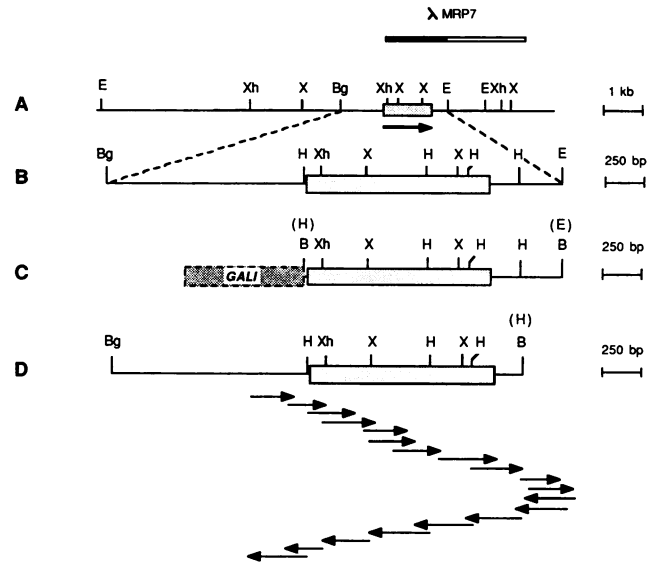

FIG. 3. Restriction map of the *MRP7* gene and its flanking regions. (A) Restriction map of the 12-kb insert in YCp50-*MRP7* (pKF5). Some of the *Bgl*II sites are not shown. The orientation of the yeast DNA insert in λ*MRP7* is shown above the map, with the solid bar indicating the fragment used in colony hybridization to isolate YCp50-*MRP7* clones. (B) Structure of the 2.9-kb *Bgl*II-*Eco*RI fragment that was derived by digesting pKF5 with *Bgl*II and *Eco*RI, isolating the 2.6- and 2.9-kb fragments, and religating. The *Bgl*II-*Eco*RI fragment from YCp50, which contains the Amp^r marker and *E. coli* origin of replication, is 2.6 kb. (C) Structure of *GAL1-MRP7* promoter fusion. The *Bam*HI sites were generated by partial *Hin*dIII digestion of pKF11 and ligation of *Bam*HI linkers (see Materials and Methods). The original sites are shown in parentheses. (D) Structure of the *MRP7* subclone in YEp13 resulting in pKF33 (see Materials and Methods). Abbreviations for restriction sites: E, *Eco*RI; B, *Bam*HI; Xh, *Xho*I; X, *Xba*I; Bg, *Bgl*II; H, *Hin*dIII. The strategy used for sequencing *MRP7* is shown below the restriction maps.

the NBRF data base revealed a short stretch of MRP7 between amino acids 28 and 112 that could be aligned to the 84-amino-acid ribosomal protein L27 of *E. coli* (EL27) (Fig. 5) (4). Short gaps in the C-terminal region of EL27 were introduced to optimize the degree of similarity. Taking into account conservative substitutions, the degree of similarity between MRP7 and EL27 was 68%, which is only slightly less than the corresponding value obtained by comparing the L27 proteins of *E. coli* and *B. stearothermophilus* (BL27) (72% [25]). Within the aligned regions, 32 of 84 amino acids (38%) were exactly conserved among MRP7, EL27, and BL27. The carboxy-terminal 259-amino-acid sequence of MRP7 showed no significant similarity to other proteins in the NBRF protein data base.

Transcript mapping by S1 protection analysis revealed two transcriptional start sites at −69 and −43 with respect to the predicted translational start site (indicated with arrows in Fig. 4; data not shown). The 420 nucleotides preceding the start of translation of MRP7 lack a perfect consensus TATA box, which is found in the 5′-flanking regions of most protein-coding genes in yeast (59). However, at positions −208 and −193, two potential TATA boxes were found (TATACACAC and TATACCAA, respectively). The intron-splicing consensus TACTAAC (30) was also lacking. Furthermore, no accumulation of *MRP7* precursor RNA was seen in Northern blots (not shown) of RNA extracted from

```
-420 CATAATTATG AAATTTTTCA GCCAGTACTC TACTTTTTCA TTTCCCTCGC TAAACAAAGA GAGTCATTTG
-350 AAACGACCCA AGGAAAGACG TAAATTGAGG TATATGTAGT GACCAGAAGA GGATAAGGAT GGCTAGAGTT
-280 TGGTGTTCAA GTTCAATTGT TTGGTTGAAT AGGTACTAAA AAGATTTTCA CACAACGGCT TCTCTGTAAT
-210 GTTATACACA CTGTTTTTAT ACCAATTATG CAAATTTGGC TGTTATGACT GGGTGTTTCG ATATCTGACG
-140 TTCCGCTATT GAATTTTAAA GCTCTTTCTT TGGAGACTCA TTCAAGATAG TTCACTGAAG AATGAAGTTG

 -70 GCTCATTGTA AAAATAAAAC TCGCCAGTTT GGGTGGTCAT AATATTTCAA GCTTAACCTA AGGTGAGAAT

   1 ATG TGG AAT CCT ATT TTA CTA GAT ACT TCT AGT TTT TCA TTC CAA AAA CAT GTG TCT
   1 Met Trp Asn Pro Ile Leu Leu Asp Thr Ser Ser Phe Ser Phe Gln Lys His Val Ser

  58 GGT GTG TTC CTT CAA GTG CGT AAT GCC ACC AAA AGG GCT GCG GGC TCG AGA ACA AGT
  20 Gly Val Phe Leu Gln Val Arg Asn Ala Thr Lys Arg Ala Ala Gly Ser Arg Thr Ser

 115 ATG AAG GAT TCT GCA GGA AGA AGG TTA GGA CCA AAA AAA TAT GAA GGC CAG GAT GTT
  39 Met Lys Asp Ser Ala Gly Arg Arg Leu Gly Pro Lys Lys Tyr Glu Gly Gln Asp Val

 172 TCC ACA GGT GAA ATT ATT ATG AGG CAA AGA GGA ACA AAG TTT TAT CCT GGA GAA AAC
  58 Ser Thr Gly Glu Ile Ile Met Arg Gln Arg Gly Thr Lys Phe Tyr Pro Gly Glu Asn

 229 GTT GGT ATA GGG AAA GAT CAT TCC ATA TTC GCC TTA GAA CCT GGT GTT GTT CGT TAC
  77 Val Gly Ile Gly Lys Asp His Ser Ile Phe Ala Leu Glu Pro Gly Val Val Arg Tyr

 286 TAT CTT GAT CCT TTT CAT CCT AAA AGA AAA TTC ATT GGT GTT GCC TTG AGG CGT GAT
  96 Tyr Leu Asp Pro Phe His Pro Lys Arg Lys Phe Ile Gly Val Ala Leu Arg Arg Asp

 343 TTG AAA CTG CCA TCT CCT CAT TTT GAG CCT ACC GTA AGG AGG TTT GGC CGT TTT GAG
 115 Leu Lys Leu Pro Ser Pro His Phe Glu Pro Thr Val Arg Arg Phe Gly Arg Phe Glu

 400 TTA ACA AAT AAA AGG GCA GCA TAT AAG GAA GAA AAT TCT ATT TCT AGA AAA GAC TAT
 134 Leu Thr Asn Lys Arg Ala Ala Tyr Lys Glu Glu Asn Ser Ile Ser Arg Lys Asp Tyr

 457 CTC GCA AAA CCT AAT ATT TTG AAG CAG TTA GAG GTC AGG GAA TCT AAA AGA AAG GAG
 153 Leu Ala Lys Pro Asn Ile Leu Lys Gln Leu Glu Val Arg Glu Ser Lys Arg Lys Glu

 514 TTA CAA GAC AAG TTG AGT AAG GTT CTT CGG GAT GAA CTT AAG TTA GAT ATA AAA GAC
 172 Leu Gln Asp Lys Leu Ser Lys Val Leu Arg Asp Glu Leu Lys Leu Asp Ile Lys Asp

 571 ATC GAA TTG GCA ACG TCA TAC TTA ATT CGT GTA AGG GCT TCC CTG AAG AAT GGA TAC
 191 Ile Glu Leu Ala Thr Ser Tyr Leu Ile Arg Val Arg Ala Ser Leu Lys Asn Gly Tyr

 628 CCC ATA GAA GAT GCA AGG TTC AAT AGT AGA TAT TAT TTA AAA GAA GAA GAG CGT TTG
 210 Pro Ile Glu Asp Ala Arg Phe Asn Ser Arg Tyr Tyr Leu Lys Glu Glu Glu Arg Leu

 685 AAG GCA AGG AGA GAA AGC TGG ACG AAT GAG AAA TTG TCG GAG AGT CTA TCC AAG ATT
 229 Lys Ala Arg Arg Glu Ser Trp Thr Asn Glu Lys Leu Ser Glu Ser Leu Ser Lys Ile

 742 GAT GAG TGT AGT GAT CTT TTG AAC TCA TCT ACA TCT TTC AAT AAC AAG CTA GAG CTA
 248 Asp Glu Cys Ser Asp Leu Leu Asn Ser Ser Thr Ser Phe Asn Asn Lys Leu Glu Leu

 799 CAC CAG TAT ATT TCG GAA CAA GAA AAA CAA GCT TTG AAG GCA AAA CTA TTA GAG GAC
 267 His Gln Tyr Ile Ser Glu Gln Glu Lys Gln Ala Leu Lys Ala Lys Leu Leu Glu Asp

 856 CTA GAA AAA TCC CAA CAC TTA GAA ACC AAA AAA GAC AAG AAT TAT ATT AAG GCG CTT
 286 Leu Glu Lys Ser Gln His Leu Glu Thr Lys Lys Asp Lys Asn Tyr Ile Lys Ala Leu

 913 TTC AAG GAC GCT TGT AAT TTT TTG ACT TTA TCT GAA GAA GTA CAT TTG CGT AGG AAA
 305 Phe Lys Asp Ala Cys Asn Phe Leu Thr Leu Ser Glu Glu Val His Leu Arg Arg Lys

 970 TAC TTA AAG TCG GTC TTC CCC GAA ACA GAT AGT ACT GTT GAA ACC AAA AGC GGG AAG
 324 Tyr Leu Lys Ser Val Phe Pro Glu Thr Asp Ser Thr Val Glu Thr Lys Ser Gly Lys

1027 AAG TCA ATT GTG TCT AGA CGT TTT GAT TAC ACC AAA AAT AAA GTT GAA GTC ATT GCT
 343 Lys Ser Ile Val Ser Arg Arg Phe Asp Tyr Thr Lys Asn Lys Val Glu Val Ile Ala

1084 AGA AGT AGG CGG GCT TTT TTG AGC AAG CTT TGA TTTTCTTTCC TTACGGCACAG TATGTACACA
 362 Arg Ser Arg Arg Ala Phe Leu Ser Lys Leu ---

1147 TATAAAATGT TTAAGAATGC CTGTAAATAT TACTATTTTG TCGCGCAAGT AGCTAAACCT TTCGTCTACA
1217 CGAAACTTAA AATTTAGTGC TACATGCAAG GGAATATGTT TATACTGTAC ATTCTCGTCT TCCTTTCATT
1287 GATGATATGC ATCGCGATTG CTTTCATTTT GTGAACAATC AATTAACTAA AATGCACAGG ATAGTAAAAT
1357 CTATATTTAC AAAAAGGCGG ATAAGCAAAC ACTGAAAATC ATAATGTTTT TTGTCACTTC GGTCCTTCAA
1427 GCTTGGTAGT TGATAAATTG TAAACTATAC ATCGTTTAAT GCGACACATA CAGCTCCCTT GTGGTGTCCA
1497 CCGTACTGTC TGACAATTTC TCTAGTTGAT AAATCCCATA ATCTCACGTA ATGATCGGAC GATGCCGTTA
1567 CTAGATACGC ACTGTCAGCA CTAAATGCGC
```

FIG. 4. Nucleotide sequence of the *MRP7* gene and the deduced amino acid sequence of the gene product. The sequence was determined by using the strategy shown in Fig. 3. The arrows indicate transcriptional start sites as determined by S1 analysis. The highly conserved domain of the MRP7 protein is underlined (shown in more detail in Fig. 5). Nucleotides and amino acids are numbered on the left. The *Hin*dIII site used to construct the *GAL1-MRP7* gene fusion (Fig. 3 and 8) is underlined. Also underlined are the putative transcription termination signals.

```
MRP7   1                    Met  Trp  Asn  Pro  Ile  Leu  Leu  Asp  Thr  Ser  Ser  Phe

MRP7  13 Ser  Phe  Gln  Lys  His  Val  Ser  Gly  Val  Phe  Leu  Gln  Val  Arg  Asn

MRP7  28 Ala  Thr  Lys  Arg  Ala  Ala  Gly  Ser  Arg  Thr  Ser  Met  Lys  Asp  Ser
EL27   1 Ala  His  Lys  Lys  Ala  Gly  Gly  Ser  Thr  Arg  Asn  Gly  Arg  Asp  Ser
BL27   1 Ala  Ser  Lys  Lys  Gly  Val  Gly  Ser  Thr  Lys  Asp  Gly  Arg  Asp  Ser

MRP7  43 Ala  Gly  Arg  Arg  Leu  Gly  Pro  Lys  Lys  Tyr  Glu  Gly  Gln  Asp  Val
EL27  16 Glu  Ala  Lys  Arg  Leu  Gly  Val  Lys  Arg  Phe  Gly  Gly  Glu  Ser  Val
BL27  16 Ile  Ala  Lys  Arg  Leu  Gly  Ala  Lys  Arg  Ala  Asp  Gly  Gln  Phe  Val

MRP7  58 Ser  Thr  Gly  Glu  Ile  Ile  Met  Arg  Gln  Arg  Gly  Thr  Lys  Phe  Tyr
EL27  31 Leu  Ala  Gly  Ser  Ile  Ile  Val  Arg  Gln  Arg  Gly  Thr  Lys  Phe  His
BL27  31 Thr  Gly  Gly  Ser  Ile  Leu  Tyr  Arg  Gln  Arg  Gly  Thr  Lys  Val  His

MRP7  73 Pro  Gly  Glu  Asn  Val  Gly  Ile  Gly  Lys  Asp  His  Ser  Ile  Phe  Ala
EL27  46 Ala  Gly  Ala  Asn  Val  Gly  Lys  Gly  Arg  Asp  His  Thr  Leu  Phe  Ala
BL27  46 Pro  Gly  Leu  Asn  Val  Gly  Arg  Gly  Arg  Asp  Asp  Thr  Leu  Tyr  Ala

MRP7  88 Leu  Glu  Pro  Gly  Val  Val  Arg  Tyr  Tyr  Leu  Asp  Pro  Phe  His  Pro
EL27  61 Lys  Ala  Asp  Gly  Lys  Val  Lys  Phe  Glu  Val  Lys  Gly   -    -   Pro
BL27  61 Lys  Ile  Asp  Gly  Ile  Val  Arg  Phe  Glu  Arg  Leu  Gly  Arg  Asp  Arg

MRP7 103 Lys   -   Arg  Lys  Phe  Ile  Gly  Val  Ala  Leu  Arg  Arg  Asp
EL27  74 Lys  Asn  Arg  Lys  Phe  Ile  Ser  Ile  Glu  Ala  Glu   -    -
BL27  76 Lys   -   Arg  Val  Ser  Val  Tyr  Pro  Val  Ser  Gln  Glu  Ala
```

FIG. 5. Similarity between MRP7 and two procaryotic ribosomal proteins. EL27 is the L27 protein from *E. coli*, and BL27 is the L27 homolog from *B. stearothermophilus*. The homology begins at amino acid 28 of MRP7 and extends through amino acid 112. Dashes indicate gaps introduced to optimize the alignment. Identities with MRP7 are boxed.

an *rna2* mutant, which fails to process intervening sequences from pre-mRNAs at the restrictive temperature (46). Together, these results suggest that, unlike many of the cytoplasmic ribosomal protein genes, *MRP7* does not contain an intervening sequence.

Several sequence elements have been proposed to be involved in transcription termination and polyadenylation in yeast. The putative polyadenylation signal AATAAA (15) was found within the *MRP7* open reading frame, beginning at nucleotide 1063. Henikoff and Cohen proposed that TTTTTATA is an important sequence for transcription termination (18) and that the 3′ mRNA sequences fit the consensus CAAGT/CTTTG (19). Although the TTTTTATA consensus was not found anywhere near the 3′ end of *MRP7*, the sequence CAAGCTTTG was found at the end of the open reading frame and included part of the stop codon (underlined in Fig. 4). Finally, Zaret and Sherman proposed a consensus of TAG. . .TATGT. . .TTT as a transcriptional termination signal (74). Two sets of these sequences were found downstream of the *MRP7* coding region (also underlined in Fig. 4). At present, we do not know whether any of these sequences actually function in transcription termination or polyadenylation of *MRP7* mRNA.

***MRP7* expression is controlled by catabolite repression.** As a first approach to understanding the regulation of *MRP7*, the steady-state levels of *MRP7* transcripts and protein were determined in [*rho*$^+$] and [*rho*^{0}] derivatives of yeast strain MH41-7B growing under full repression in 5% glucose, intermediate repression in 2% galactose, and, for the [*rho*$^+$] strain, under full derepression in 2% glycerol plus 2% ethanol. The results of the Northern and Western blot analyses are shown in Fig. 6. The levels of the 1.5-kb *MRP7* transcripts were relatively invariant with respect to either the degree of repression or the mitochondrial genetic background. In contrast, the levels of the MRP7 protein were approximately ninefold higher in derepressed than in repressed cells. It is also noteworthy that the MRP7 protein accumulated at normal levels in the [*rho*^{0}] cells, which are totally devoid of mitochondrial rRNAs and are therefore unable to assemble ribosomal subunits. These results implicate a strong posttranscriptional component in the response of *MRP7* to catabolite repression. Furthermore, since the MRP7 protein appears to be stable in the absence of the 21S rRNA, the control point in this regulation may be at the level of protein synthesis, not degradation.

Gene dosage compensation of *MRP7*. Increasing the dosage

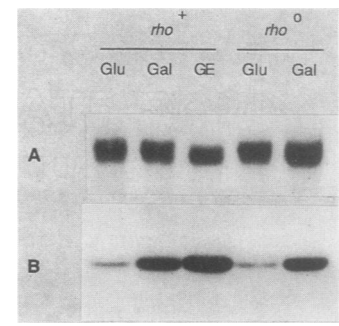

FIG. 6. Catabolite repression of *MRP7*. Yeast strains MH41-7B [*rho*$^+$] and MH41-7B [*rho*^{0}] were grown in rich medium with one of the following as a sole carbon source: 5% glucose (Glu), 2% galactose (Gal), or 2% glycerol plus 2% ethanol (GE). Total RNA (20 μg) or total cell protein (100 μg) was loaded in each lane. (A) Northern blots. (B) Western blots (with alkaline hydrolysis and trichloroacetic acid precipitation for protein preparation and nonfat dry milk to saturate blot). Lanes are labeled according to mitochondrial genetic background and carbon source used for growth.

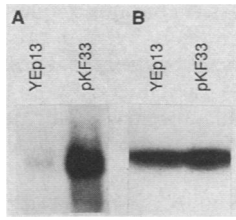

FIG. 7. Gene dosage compensation of *MRP7*. 22-2D [*rho*$^+$] was transformed with pKF33 (Fig. 3D) or YEp13 as a control. Transformants were grown on minimal medium with 2% glycerol plus 2% ethanol, selecting for LEU$^+$. Total RNA (20 μg) (A) or total protein (100 μg) (B) was loaded in each lane. The probe for the Northern blot (A) was the 600-bp *Xba*I fragment from the *MRP7* coding region. Proteins were prepared by breakage with glass beads and solubilization with SDS. Nonfat dry milk was used to saturate the Western blot (B).

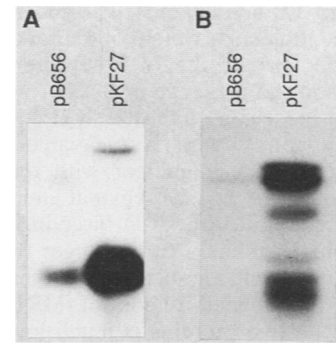

FIG. 8. Expression of *MRP7* under the direction of the *GAL1* promoter. 22-2D [*rho*$^+$] was transformed with pKF27 (Fig. 3C) or pB656 as a control. Transformants were grown on minimal medium with 2% galactose as a carbon source, selecting for Ura$^+$. Total RNA (20 μg) (A) or total protein (100 μg) (B) was loaded in each lane. The probe for the Northern blot (A) was the 600-bp *Xba*I fragment from the *MRP7* coding region. Proteins were prepared by breakage with glass beads and solubilization with SDS. Nonfat dry milk was used to saturate the Western blot (B). The immunoreactive band migrating above the mature MRP7 protein in the Western blot is probably the unprocessed MRP7 precursor.

of genes for *E. coli* and yeast cytoplasmic ribosomal proteins led to the discovery of posttranscriptional events that compensate for higher gene copy number and thereby maintain balanced production of the ribosomal proteins (16, 31, 40, 44, 66). To determine whether the expression of *MRP7* is also gene dosage compensated, we increased the copy number of *MRP7* by transforming yeast strain 22-2D [*rho*$^+$] with pKF33, a multicopy plasmid constructed by ligating a 2.6-kb *Bgl*II-*Bam*HI fragment carrying *MRP7* (Fig. 3D) into the *Bam*HI site of YEp13. The steady-state level of the *MRP7* transcript was approximately 20-fold higher in cells transformed with pKF33 than in cells transformed with the YEp13 vector without an insert (Fig. 7A). The increase in the RNA level was in agreement with the 20-fold increase in gene dosage determined by a quantitative Southern blot (data not shown). In contrast, there was no significant increase in the level of the MRP7 protein (Fig. 7B). These results are qualitatively similar to those obtained for most of the other ribosomal proteins that have been examined; transcription increases in proportion to gene dosage with little if any excess accumulation of the ribosomal protein. Although kinetic labeling experiments will be required to distinguish among the different posttranscriptional events that could account for the dosage compensation of *MRP7*, it is worth noting again that the apparent stability of MRP7 in the absence of subunit assembly argues in favor of a compensatory effect on synthesis rather than on protein turnover.

Overexpression of MRP7 from a *GAL1-MRP7* fused gene. To evaluate further the ability of MRP7 to accumulate in excess of the amount needed for ribosome assembly, we placed *MRP7* under the control of the *GAL1* promoter (Fig. 3C) in a multicopy vector. In this construction, the upstream region of *MRP7* beginning at –22 was replaced by a 0.8-kb fragment of *GAL1* sequence, including the UAS elements, TATA box, transcriptional start sites, and 55 nucleotides of the 5′ leader to within 4 nucleotides of the *GAL1* translational start (5). The transcript and protein levels were compared in cells grown in either 5% glucose or 2% galactose. Both the mRNA and the protein were dramatically induced by growth in galactose in the strain carrying the *GAL1-MRP7* fused gene (data not shown). Within the limits of the assays, the overexpression of the MRP7 protein was roughly proportional to that of the mRNA compared with these levels in the control strain (approximately 30-fold, Fig. 8). Although some increase in the degradation of excess MRP7 at high levels of expression was suggested by the appearance of short immunoreactive polypeptides in galactose-induced cells (Fig. 8B, lane 2), MRP7 did not appear to be inherently unstable when it is present in large excess in a presumably unassembled form. In fact, a putative precursor to MRP7 also accumulated in this strain, as can be seen by the immunoreactive band with a slightly slower migration than mature MRP7. These results raise important questions about why the protein is overproduced with transcripts derived from the *GAL1-MRP7* fused gene but not with transcripts from extra copies of the *MRP7* gene itself. In this regard, it should be noted that the *GAL1-MRP7* gene fusion produces transcripts in which the nucleotides from −22 to the 5′ ends of the normal *MRP7* mRNA at −43 and −69 are replaced by 55 nucleotides of the *GAL1* 5′ leader. The possible involvement of the 5′ end of the mRNA in *MRP7* regulation is under investigation.

DISCUSSION

Immunological screening of a λgt11 yeast genomic library with monoclonal antibodies enabled us to identify *MRP7*, the nuclear gene for a large-subunit protein of the mitochondrial ribosome. Gene disruption of *MRP7* has shown that the protein product is essential for mitochondrial translation. Haploid strains carrying only a disrupted allele of *MRP7* are unable to grow on nonfermentable carbon sources and appear to be genotypically [*rho*$^-$] in crosses to strains with known mitochondrial genetic defects. A linkage between mitochondrial translation and the maintenance of wild-type mitochondrial DNA in *Saccharomyces cerevisiae* was suggested earlier by the high frequency of [*rho*$^-$] in cultures grown in the presence of antibiotics that block translation on mitochondrial ribosomes (2, 68, 70). More recently, Myers et al. (38, 39) corroborated these results by creating stringent mutations in genes for essential components of the mitochondrial translation system. The results presented here provide another example in which disruption of mitochondrial translation results in loss of wild-type mtDNA.

A functional copy of *MRP7* was isolated by colony hybridization of a YCp50 yeast genomic library. The plasmid-borne copy of *MRP7* was able to complement the genomic disruption. Analysis of the *MRP7* sequence revealed an open

reading frame of 371 amino acids. The specified polypeptide had a calculated molecular weight of 43,223, which is slightly higher than the size of the MRP7 protein estimated by electrophoresis in the presence of SDS. This discrepancy is consistent with the assumption that MRP7 is translated as a precursor polypeptide with a cleavable amino-terminal leader sequence. Since alignment with the *E. coli* large-subunit ribosomal protein L27 began at amino acid 28 of the MRP7 sequence, a mitochondrial targeting sequence in an MRP7 precursor is unlikely to be longer than 27 residues. There is 49% amino acid identity between the 84 amino acids of EL27 and the aligned region of MRP7. The overall relatedness of the two proteins is much lower (11%), however, mainly because there is no alignment with the carboxy-terminal 259 residues of MRP7. Considering only the regions where alignments are clear, the amino acid conservation between MRP7 and EL27 is the highest described to date for a comparison between a mitochondrial and an *E. coli* ribosomal protein. In fact, only two of the four yeast mitochondrial ribosomal proteins examined so far show significant sequence relatedness to a bacterial counterpart (39, 42). A putative mitochondrial r-protein in *Drosophila melanogaster* is related to *E. coli* S12 with 41% amino acid identity in the aligned region (49).

The high level of sequence conservation between L27 and MRP7 suggests that many of the functional properties of L27 will hold true for MRP7. L27 has been identified as a constituent of the peptidyl transferase center on the basis of cross-linking experiments with either peptidyl- or aminoacyl-tRNA affinity labels and also with derivatives of antibiotics believed to act at the peptidyl transferase center (41). Immunoelectron microscopy has localized L27 on the central protuberance of the large subunit (29). Antibodies that bind to L27 prevent reassociation of subunits to form the 70S particle (37), and L27 can be cross-linked to S9 of the small subunit (64), indicating a location at or near the subunit interface of the ribosome. As a member of the "late assembly group," L27 is not required in the early stages of assembly of the 50S particle (44), nor does it bind to free 23S rRNA. It remains to be determined whether any of these characteristics of L27 are in fact embodied in MRP7.

While the relatedness to L27 implicates MRP7 as a component of the peptidyl transferase center, most of the polypeptide bears no obvious sequence relatedness to known ribosomal proteins and its possible functional significance remains obscure. A similar distinction between conserved and unique elements has recently been seen in the yeast mitochondrial RNA polymerase, which is related to the RNA polymerases of T3, T7, and SP6 bacteriophages (35). In the yeast polymerase, the carboxy-terminal two-thirds of the protein contains the major conserved domains, and the amino-terminal third, a stretch of about 400 amino acids, does not align with the phage molecules. It seems reasonable to ascribe common functions to the conserved domains and organelle-specific functions to the additional, apparently unique sequences present in the MRP7 and the mitochondrial RNA polymerase molecules. Such composite proteins could have evolved along several different paths, but one of the more attractive possibilities is that multifunctional organellar proteins originated from fusions between smaller genes encoding proteins with separate functions. Gene fusion events have been proposed as the evolutionary origin of the *TRP5*, *HIS4*, and *ADE3* gene products in yeast, each of which is a single protein with multiple enzymatic activities (24). Also relevant in this regard is the recent discovery that MRP15, a nucleus-encoded mitochondrial ribosomal protein in *Neurospora crassa*, contains sequence similarities to S4 and S5, two functionally related proteins of the *E. coli* ribosome (J. E. Heckman, personal communication). Multifunctionality, if it is a relatively common feature of mitochondrial ribosomal proteins, could be an explanation for why these proteins are on average larger than their counterparts in *E. coli*.

Since *E. coli* mutants lacking L27 are viable, albeit with severe impairment of growth, L27 is not absolutely essential for ribosome assembly and function (7). In contrast to this, we have classified MRP7 as an essential protein on the basis of our characterization of *MRP7* null mutants, which are completely devoid of mitochondrial protein synthesis. It should be noted, however, that we have not determined whether the loss of protein synthesis is a direct effect of the MRP7 deficiency or an indirect consequence of the conversion to [rho^-]. Our experiments only show that *MRP7* is essential for levels of mitochondrial translation above the threshold necessary for maintenance of [rho^+] mtDNA. The molecular basis for the linkage between mitochondrial translation and the maintenance of [rho^+] mtDNA has not been established with respect to either how much protein synthesis is required or what the essential product(s) might be.

The biosynthesis of ribosomes is tightly coordinated in procaryotes and eucaryotes. In *E. coli*, translational feedback regulation appears to be a major factor in balancing the synthesis of ribosomal proteins to rRNA. Several operons are autoregulated at the translational level by regulatory ribosomal proteins. The feedback is operon specific and mediated through the interaction of the regulatory ribosomal protein with its own mRNAs. Thus, when these operons are amplified in the cell, mRNA levels increase proportionally, but there is no significant increase in the synthesis of many of the ribosomal proteins encoded by the operon. Interestingly, L27 is apparently not subject to translational autoregulation (60); the rate of L27 synthesis increased by a factor of 1.75 in merodiploids, with a twofold increase in the dosage of the gene for L27.

Posttranscriptional events are also important in balancing the accumulation of cytoplasmic ribosomal proteins in yeast. The basic observation is similar to that described above for *E. coli*; amplification of genes for ribosomal proteins leads to overexpression of the mRNAs but with little if any accumulation of excess protein. While there is evidence for translational modulation in the synthesis of some of these proteins (43, 67), the accumulation of others is clearly controlled by rapid degradation of excess copies of the proteins (1, 12, 33, 67). Autoregulation seems not to be a factor, with the possible exception of feedback of RPL32 on the splicing of its own pre-mRNA (8).

Our analyses of the steady-state levels of mRNA and protein in response to increased gene dosage and catabolite repression provide convincing evidence for posttranscriptional regulation of *MRP7* expression. The response to catabolite repression is characterized by a ninefold change in the level of the protein and little, if any, change in the level of the mRNA. Significantly, the imbalance between protein and mRNA occurs in wild-type cells as part of a normal physiological response. When imbalances were created by increasing the copy number of *MRP7* approximately 20-fold, there was a corresponding increase in the level of the mRNA but only a marginal increase in the amount of the MRP7 protein in the cells. Thus, accumulation of MRP7 is compensated for during increases in gene dosage, as are most other ribosomal proteins that have been examined in this manner. Although it would be interesting to know, for comparison,

whether oversynthesis of L27 leads to its accumulation in merodiploid *E. coli* strains (60), we are not aware of any definitive information about the steady-state levels of L27 in these strains.

Although pulse and pulse-chase labeling experiments will be required to determine conclusively whether the levels of MRP7 are controlled at the level of synthesis or degradation, two experimental observations support the idea that MRP7 is regulated translationally. First, the protein accumulates at normal levels in the absence of ribosome assembly, an unusual property for ribosomal proteins, which characteristically do not accumulate as free proteins. Second, MRP7 accumulates in excess when it is translated from a *GAL1-MRP7* hybrid mRNA. These results strongly suggest that the MRP7 protein is relatively stable in an unassembled form and that it accumulates in proportion to its rate of synthesis. As a working model, we propose that transcription from *MRP7* is constitutive and that catabolite repression and gene dosage compensation are mediated through translational controls.

The regulatory properties of *MRP7* appear to differ from those of the three other nuclear genes for mitochondrial ribosomal proteins that have been characterized thus far. Although all four genes respond to catabolite repression to about the same extent, *MRP1*, *MRP2*, and *MRP13* are transcriptionally regulated (39, 42), whereas MRP7 levels are modulated posttranscriptionally. The significance of this difference may be elucidated through further analysis of *MRP7* and characterization of additional genes for mitochondrial ribosomal proteins.

ACKNOWLEDGMENTS

We thank David Perry for the monoclonal antibody to MRP7, Lynn Ching for her help in sequencing, Rick Young for providing the λgt11 genomic library, Mark Rose for the YCp50 genomic library, Kelly Tatchell for pRa545, and Gerry Fink and Molly Fitzgerald-Hayes for the gifts of yeast strains and plasmids. We are grateful to Maurille Fournier and Robert Zimmermann for critical reading of the manuscript.

This work was supported by National Science Foundation grant DCB-8502553 to T.L.M.

LITERATURE CITED

1. **Abovich, N., L. Gritz, L. Tung, and M. Rosbash.** 1985. Effect of *RP51* gene dosage alterations on ribosome synthesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**:3429–3435.
2. **Carnevali, F., L. Leoni, G. Morpurgo, and G. Conti.** 1971. Induction of cytoplasmic "petite" mutation by antibacterial antibiotics. Mutat. Res. **12**:357–363.
3. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
4. **Chen, R., L. Mende, and U. Arfsten.** 1975. The primary structure of protein L27 from the peptidyl-tRNA binding site of *Escherichia coli* ribosomes. FEBS Lett. **59**:96–99.
5. **Citron, B. A., and J. E. Donelson.** 1984. Sequence of the *Saccharomyces GAL* region and its transcription in vivo. J. Bacteriol. **158**:269–278.
6. **Curgy, J. J.** 1985. The mitoribosomes. Biol. Cell **54**:1–38.
7. **Dabbs, E. R., R. Hasenbank, B. Kaster, K.-H. Rak, B. Wartusch, and G. Stoffler.** 1983. Immunological studies of *Escherichia coli* mutants lacking one or two ribosomal proteins. Mol. Gen. Genet. **192**:301–308.
8. **Dabeva, M. D., M. A. Post-Beittenmiller, and J. R. Warner.** 1986. Autogenous regulation of splicing of the transcript of a yeast ribosomal protein gene. Proc. Natl. Acad. Sci. USA **83**: 5854–5857.
9. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S rDNA. Plasmid **13**:31–40.
10. **Daum, G., P. Boehni, and G. Schatz.** 1982. Import of proteins into mitochondria: cytochrome b_2 and cytochrome *c* peroxidase are located in the intermembrane space of yeast mitochondria. J. Biol. Chem. **257**:13028-13033.
11. **Douglas, M. G., M. T. McCammon, and A. Vassarotti.** 1986. Targeting proteins into mitochondria. Microbiol. Rev. **50**:166–178.
12. **El Baradi, T. T. A. L., C. A. F. M. van der Sande, W. H. Mager, H. A. Raue, and R. J. Planta.** 1986. The cellular level of yeast ribosomal protein L25 is controlled principally by rapid degradation of excess protein. Curr. Genet. **10**:733–739.
13. **Favaloro, J., R. Treisman, and R. Kamen.** 1980. Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S1 gel mapping. Methods Enzymol. **65**:718–749.
14. **Faye, G., and F. Sor.** 1977. Analysis of mitochondrial ribosomal proteins of *Saccharomyces cerevisiae* by two dimensional polyacrylamide gel electrophoresis. Mol. Gen. Genet. **155**:27–34.
15. **Fitzgerald, M., and T. Shenk.** 1981. The sequence 5′-AAUAAA-3′ forms part of the recognition site for polyadenylation of late SV40 mRNAs. Cell **24**:251–260.
16. **Gourse, R. L., R. A. Sharrock, and M. Nomura.** 1986. Control of ribosome synthesis in *Escherichia coli*, p. 766–788. *In* B. Hardesty and G. Kramer (ed.), Structure, function, and genetics of ribosomes. Springer-Verlag, New York.
17. **Hanahan, D., and M. Meselson.** 1980. Plasmid screening at high colony density. Gene **10**:63–67.
18. **Henikoff, S., and E. H. Cohen.** 1984. Sequences responsible for transcription termination on a gene segment in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1515–1520.
19. **Henikoff, S., J. D. Kelly, and E. H. Cohen.** 1983. Transcription terminates in yeast distal to a control sequence. Cell **33**:607–614.
20. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
21. **Hurt, E. C., and A. P. G. M. van Loon.** 1986. How proteins find mitochondrial and intramitochondrial compartments. Trends Biol. Sci. **11**:204–207.
22. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
23. **Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene. Anal. Technol. **1**:3–8.
24. **Jones, E. W., and G. R. Fink.** 1982. Regulation of amino acid and nucleotide biosynthesis in yeast, p. 181–299. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Kimura, M., and C. K. Chow.** 1984. The complete amino acid sequences of ribosomal proteins L17, L27, and S9 from *Bacillus stearothermophilus*. FEBS Lett. **139**:225–234.
26. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**:1–45.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
28. **Lake, J. A.** 1985. Evolving ribosome structure: domains in archaebacteria, eubacteria, eocytes and eukaryotes. Annu. Rev. Biochem. **54**:507–530.
29. **Lake, J. A., and W. A. Strycharz.** 1981. Ribosomal proteins L1, L17 and L27 from *Escherichia coli* localized at single sites on the large subunit by immune electron microscopy. J. Mol. Biol. **153**:979–992.
30. **Langford, C. J., and D. Gallwitz.** 1983. Evidence for an intron-contained sequence required for the splicing of yeast RNA polymerase II transcripts. Cell **33**:519–527.

31. **Lindahl, L., and J. M. Zengel.** 1986. Ribosomal genes in *Escherichia coli*. Annu. Rev. Genet. **20**:297–326.
32. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
33. **Maicas, E., F. G. Pluthero, and J. D. Friesen.** 1988. The accumulation of three yeast ribosomal proteins under conditions of excess mRNA is determined primarily by fast protein decay. Mol. Cell. Biol. **8**:169–175.
34. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Masters, B. S., L. L. Stohl, and D. A. Clayton.** 1987. Yeast mitochondrial RNA polymerase is homologous to those encoded by bacteriophages T3 and T7. Cell **51**:89–99.
36. **Messing, J.** 1983. The new M13 vectors for cloning. Methods Enzymol. **101**:20–78.
37. **Morrison, C. A., R. A. Garrett, H. Zeichhardt, and G. Stoffler.** 1973. Proteins occurring at, or near, the subunit interface of *E. coli* ribosomes. Mol. Gen. Genet. **127**:359–368.
38. **Myers, A. M., L. K. Pape, and A. Tzagoloff.** 1985. Mitochondrial protein synthesis is required for maintenance of intact mitochondrial genomes in *Saccharomyces cerevisiae*. EMBO J. **4**: 2087–2092.
39. **Myers, A. M., M. D. Crivellone, and A. Tzagoloff.** 1987. Assembly of the mitochondrial membrane system: *MRP1* and *MRP2*, two yeast nuclear genes coding for mitochondrial ribosomal proteins. J. Biol. Chem. **262**:3388–3397.
40. **Nomura, M., R. Gourse, and G. Baughman.** 1984. Regulation of the synthesis of ribosomes and ribosomal components. Annu. Rev. Biochem. **53**:75–118.
41. **Ofengand, J., J. Ciesiolka, R. Denman, and K. Nurse.** 1986. Structural and functional interactions of the tRNA-ribosome complex, p. 473–494. *In* B. Hardesty and G. Kramer (ed.), Structure, function, and genetics of ribosomes. Springer-Verlag, New York.
42. **Partaledis, J. A., and T. L. Mason.** 1988. Structure and regulation of a nuclear gene in *Saccharomyces cerevisiae* that specifies MRP13, a protein of the small subunit of the mitochondrial ribosome. Mol. Cell. Biol. **8**:3647–3660.
43. **Pearson, N. J., H. M. Fried, and J. R. Warner.** 1982. Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell **29**:347–355.
44. **Planta, R. J., W. H. Mager, R. J. Leer, L. P. Woudt, H. A. Raue, and T. T. A. L. El Baradi.** 1986. Structure and expression of ribosomal protein genes in yeast, p. 699–718. *In* B. Hardesty and G. Kramer (ed.), Structure, function, and genetics of ribosomes. Springer-Verlag, New York.
45. **Rohl, R., and K. H. Nierhaus.** 1982. Assembly map of the large subunit (50S) of *Escherichia coli* ribosomes. Proc. Natl. Acad. Sci. USA **79**:729–733.
46. **Rosbash, M., P. K. W. Harris, J. L. Woolford, Jr., and J. L. Teem.** 1981. The effect of temperature-sensitive RNA mutants on the transcription products from cloned ribosomal protein genes of yeast. Cell **24**:679–686.
47. **Rose, M. D., and G. R. Fink.** 1987. *KAR1*, a gene required for function of both intranuclear and extranuclear microtubules in yeast. Cell **48**:1047–1060.
48. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
49. **Royden, C. S., V. Pirrotta, and L. Y. Jan.** 1987. The *tko* locus, site of a behavioral mutation in *D. melanogaster*, codes for a protein homologous to prokaryotic ribosomal protein S12. Cell **51**:165–173.
50. **Sanger, F., and A. R. Coulson.** 1978. The use of thin acrylamide gels for DNA sequencing. FEBS Lett. **87**:107–110.
51. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5469.
52. **Seth, A.** 1984. A new method for linker ligation. Gene. Anal. Technol. **1**:99–103.
53. **Sharp, P. M., T. M. F. Tuohy, and K. R. Mosurski.** 1986. Codon usage in yeast: cluster analysis clearly differentiates highly and lowly expressed genes. Nucleic Acids Res. **14**:5125–5143.
54. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
55. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
56. **Snyder, M., S. Elledge, and R. W. Davis.** 1986. Rapid mapping of antigenic coding regions and constructing insertion mutations in yeast genes by mini-Tn*10* "transplason" mutagenesis. Proc. Natl. Acad. Sci. USA **83**:730–734.
57. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
58. **Sprague, G. F., Jr., R. Jensen, and I. Herskowitz.** 1983. Control of yeast cell type by the mating type locus: positive regulation of the alpha-specific *STE3* gene by the *MATα1* product. Cell **32**: 409–415.
59. **Struhl, K.** 1987. Promoters, activator proteins, and the mechanism of transcriptional initiation in yeast. Cell **49**:295–297.
60. **Takata, R.** 1978. Genetic studies of the ribosomal proteins in *Escherichia coli* XI. Mol. Gen. Genet. **160**:151–155.
61. **Tatchell, K., D. T. Chaleff, D. DeFeo-Jones, and E. M. Scolnick.** 1984. Requirement of either of a pair of *ras*-related genes of *Saccharomyces cerevisiae* for spore viability. Nature (London) **309**:523–527.
62. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
63. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
64. **Traut, R. R., J. M. Lambert, G. Boileau, and J. W. Kenny.** 1980. Protein topography of *Escherichia coli* ribosomal subunits as inferred from protein crosslinking, p. 89–110. *In* G. Chambliss, G. R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura (ed.), Ribosomes: structure, function, and genetics. University Park Press, Baltimore.
65. **Tzagoloff, A., and A. M. Myers.** 1986. Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. **55**:249–285.
66. **Warner, J. R., E. A. Elion, M. D. Dabeva, and W. F. Schwindinger.** 1986. The ribosomal genes of yeast and their regulation, p. 719–732. *In* B. Hardesty and G. Kramer (ed.), Structure, function, and genetics of ribosomes. Springer-Verlag, New York.
67. **Warner, J. R., G. Mitra, W. F. Schwindinger, M. Studeny, and H. M. Fried.** 1985. *Saccharomyces cerevisiae* coordinates accumulation of yeast ribosomal proteins by modulating mRNA splicing, translational initiation, and protein turnover. Mol. Cell. Biol. **5**:1512–1521.
68. **Weislogel, P. O., and R. A. Butow.** 1970. Low temperature and chloramphenicol induction of respiratory deficiency in a cold-sensitive mutant of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **67**:52–58.
69. **Wilbur, W. J., and D. J. Lipman.** 1983. Rapid similarity searches of nucleic acid and protein data banks. Proc. Natl. Acad. Sci. USA **80**:726–730.
70. **Williamson, D. H., N. G. Maroukas, and D. Wilkie.** 1971. Induction of the cytoplasmic petite mutation in *Saccharomyces cerevisiae* by the antibacterial antibiotics erythromycin and chloramphenicol. Mol. Gen. Genet. **111**:209–223.
71. **Yaffe, M. P., and G. Schatz.** 1984. Two nuclear mutations that block mitochondrial protein import in yeast. Proc. Natl. Acad. Sci. USA **81**:4819–4823.
72. **Young, R. A., and R. W. Davis.** 1983. Yeast RNA polymerase II genes: isolation with antibody probes. Science **222**:778–782.
73. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80**:1194–1198.
74. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.