17. Z. Chen and D. F. Klessig, unpublished data.
18. M. W. Sutherland, *Physiol. Mol. Plant Pathol.* **93**, 79 (1991).
19. D. J. Bradley, P. Kjellborn, C. J. Lamb, *Cell* **70**, 21 (1992).
20. R. Schreck and P. A. Baeuerle, *Trends Cell Biol.* **1**, 39 (1991).
21. Y. Devary, R. A. Gottlieb, L. F. Lau, M. Karin, *Mol. Cell Biol.* **11**, 2804 (1991).
22. J. Hennig, J. Malamy, D. F. Klessig, unpublished data.
23. V. Raz and R. Fluhr, *Plant Cell* **5**, 523 (1993).
24. G. N. Rao, B. Lassegue, K. K. Griendling, R. W. Alexander, B. C. Berk, *Nucleic Acids Res.* **21**, 1259 (1993).
25. Substantial proportions of catalase activity in crude extracts from cucumber, tomato, and *Arabidopsis* were inhibited by SA, whereas catalases purified from human erythrocytes and dog, bovine, or mouse livers were insensitive to SA (P. Sanchez-Casas, J. Ricigliano, D. F. Klessig, unpublished data.)
26. J. C. Stein and J. B. Nasrallah, *Plant Physiol.* **101**, 1103 (1993); C. Chang, S. F. Kwok, A. B. Bleecker, E. M. Meyerowitz, *Science* **262**, 539 (1993); J. J. Kieber, M. Rothenberg, G. Roman, K. Feldmann, J. R. Ecker, *Cell* **72**, 427 (1993); A. Rück, K. Palme, M. A. Venis, R. M. Napier, H. H. Felle, *Plant J.* **4**, 41 (1993); R. M. Napier and M. A. Venis, *Trends Biochem. Sci.* **16**, 72 (1991); P. V. Prasad and A. M. Jones, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5479 (1991); O. N. Kulaeva, N. N. Karavaiko, I. E. Moshkov, S. Y. Selivankina, G. V. Novikova, *FEBS Lett.* **261**, 410 (1990).
27. G. Weissmann, *Sci. Am.* **264**, 84 (January 1991).
28. Quantification of H_2O_2 was performed by the chemiluminescence reaction with luminol as described [E. Warm and G. G. Laties, *Phytochem.* **21**, 827 (1982)] with some modifications. Test solution (50 μl) and luminol (50 μl of 0.5 mM in 0.2 N NH_4OH, pH 9.5) were added to 0.8 ml of 0.2 N NH_4OH, pH 9.5 in a test tube. After placing the tube in the measuring chamber, the assay was initiated with automatic injection into the mixture of 100 μl of 0.5 mM $K_3Fe(CN)_6$ in 0.2 N NH_4OH, pH 9.5. Measurements were integrated over 5-s periods. H_2O_2 concentration was calculated from the standard curve constructed with known amounts of H_2O_2. Oxygen production and its inhibition by SA were also measured with an oxygen electrode to ensure that catalase, but not peroxidase, activity was being assayed.
29. P. Abad, A. Marais, L. Cardin, A. Poupet, M. Ponchet, *Antiviral Res.* **9**, 315 (1988); H. M. Doherty, R. R. Selvendran, D. J. Bowles, *Physiol. Mol. Plant Pathol.* **33**, 377 (1988).
30. We thank members of the lab, particularly D. Dempsey and J. Tonkyn, for their critical reading of this manuscript, and E. Lam for providing the tobacco cDNA library. Supported in part by grants DCB-9003711 from the National Science Foundation and 92-37301-7599 from the U.S. Department of Agriculture.

3 September 1993; accepted 10 November 1993

Functional Requirement of a Site-Specific Ribose Methylation in Ribosomal RNA

Karen Sirum-Connolly and Thomas L. Mason*

The product of the *PET56* nuclear gene of *Saccharomyces cerevisiae* was shown to be required for ribose methylation at a universally conserved nucleotide in the peptidyl transferase center of the mitochondrial large ribosomal RNA (21*S* rRNA). Cells reduced in this activity were deficient in formation of functional large subunits of the mitochondrial ribosome. The purified Pet56 protein catalyzed the site-specific formation of 2′-*O*-methylguanosine on in vitro transcripts of both mitochondrial 21*S* rRNA and *Escherichia coli* 23*S* rRNA. These results provide evidence for an essential modified nucleotide in rRNA.

Peptidyl transferase is an intrinsic activity of the large ribosomal subunit that catalyzes peptide bond formation in protein synthesis. The sequence and structure of the rRNA in the peptidyl transferase center are highly conserved (*1*), and there is abundant evidence supporting the possibility that rRNA participates in the peptidyl transferase reaction. Most notably, peptidyl transferase activity has been shown to be resistant to protein extraction procedures, consistent with the hypothesis that the rRNA itself is catalytically active (*2*).

The production of mature rRNA involves transcription, nucleolytic processing, and posttranscriptional modification of nucleotides. Although no clear functional role has been demonstrated for any of the modified nucleotides, their importance is suggested by models of the *E. coli* ribosome in which the modifications are clustered around the mRNA-tRNA-peptide complex in the catalytic center of the ribosome (*3*).

K. Sirum-Connolly, Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003.
T. L. Mason, Department of Biochemistry and Molecular Biology, and the Graduate Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.
*To whom correspondence should be addressed.

Of the 19 identified posttranscriptional modifications in the 23*S* rRNA of *E. coli*, three are ribose methylations at the highly conserved positions Gm2251, Cm2498, and Um2552 in the peptidyl transferase region of domain V (*4*). Mitochondrial large rRNAs are apparently devoid of base methylations but contain as many as three ribose methylations. The mitochondrial large rRNA from hamster contains three ribose-methylated nucleotides at positions corresponding to the universally conserved nucleotides G2251, U2552, and G2553 in the *E. coli* 23*S* rRNA (*5*). The mitochondrial large rRNAs of yeast and *Neurospora* have about two ribose methylations, but the identity of the modified nucleotides has not been established (*6*). The retention of ribose-methylated nucleotides in the otherwise minimally modified mitochondrial rRNAs suggests an essential role for these modifications in ribosome assembly or function, or both. Here we show that the *PET56* gene of *S. cerevisiae* encodes a site-specific rRNA ribose methyltransferase that is required for the formation of functional mitochondrial ribosomes.

The *PET56* gene, required for mitochondrial function, was discovered because it is adjacent to and divergently transcribed from *HIS3*, a histidine biosynthetic gene in the nuclear genome of *S. cerevisiae* (*7*, *8*). Sequence analysis of *PET56* revealed a long open reading frame specifying a basic [isoelectric point (pI) = 10.08], 412–amino acid (46 kD) polypeptide (*9*). The deduced amino acid sequence of Pet56p has 50 to 55% similarity to a 23*S* rRNA ribose methylase from two species of *Streptomyces* (*10*). The *Streptomyces* enzymes form 2′-*O*-methyladenosine at position A1067 of the 23*S* rRNA, rendering the ribosome resistant to the antibiotic thiostrepton, which is synthesized by these organisms (*11*). There was no significant sequence relatedness between Pet56p and several base-modifying methylases (*10*).

Many laboratory yeast strains carry the *his3-Δ200* mutation, a 1036–base pair deletion that removes the entire *HIS3* coding region and part of an AT-rich promoter region that is important for transcription of both *HIS3* and *PET56*. Besides causing histidine auxotrophy, this deletion decreases the transcription of *PET56* by ~80% (*7*). We observed that *his3-Δ200* mutants had a slow-growth phenotype on nonfermentable carbon sources (YPGE) (*12*) at 30°C and were respiration-deficient when grown at 18°C (*13*), presumably because of reduced expression of *PET56*. In addition, the presence of the *his3-Δ200* allele markedly enhanced the leaky respiration-deficient phenotype of a null allele of *MRP49* (*13*), the nuclear gene for a 16-kD protein in the 54*S* subunit of the mitochondrial ribosome (*14*).

We compared the yields of mitochondrial ribosomal subunits obtained by sucrose gradient centrifugation of mitochondrial lysates from the otherwise isogenic *HIS3* and *his3-Δ200* strains. The yield of large subunits from the *his3-Δ200* strain was only 22 to 29% of that from the *HIS3* strain, whereas the yield of small subunits was 82 to 97% (Fig. 1A). Thus, the mutation appears to affect the synthesis or stability of the large subunit. However, Pet56p did not cosedi-

ment with either subunit in sucrose gradient analyses of the *HIS3* strain (Fig. 1B) and was below the level of detection in the *his3-Δ200* strain (*15*). Together, these results indicate that Pet56p, although not a ribosomal protein, is important for the normal accumulation of the mitochondrial large ribosomal subunit.

A requirement for *PET56* function in the formation of the mitochondrial ribosome is also consistent with the properties of haploid *pet56* null mutants generated by gene disruption. We found that *pet56::URA3* mutants were unable to grow on YPGE and had converted quantitatively from ρ^+ to ρ^- or ρ^0 cytoplasmic petites (*15*). This instability of mitochondrial DNA is characteristic of mutants that have lost the function of a gene essential for the mitochondrial translational apparatus (*16, 17*).

To compare the methylation state of 21S rRNAs in the presence and absence of *PET56* function, we introduced the *pet56::URA3* allele into a cytoplasmic petite strain, COP161 U7 F11 ρ^-, that is known to express the 21S rRNA (*18*). Northern (RNA) blot analysis showed that the loss of *PET56* function did not affect the overall expression of the 21S rRNA (*15*), which involves 3′ end processing and, in this strain, removal of the *omega* intron (*18*). We then probed ribose methylation of the rRNA at conserved methylation sites by primer extension in an assay that is based on the resistance to alkaline hydrolysis of phosphodiester bonds on the 3′ side of 2′-O-ribose–methylated nucleotides (*19*). Briefly, extension by reverse transcriptase of appropriately positioned ^{32}P-labeled DNA oligonucleotide primers in reactions containing partially hydrolyzed rRNA as template should produce a ladder of ^{32}P-labeled extension products with gaps indicating the positions of cleavage-resistant phosphodiester bonds.

For *E. coli* 23S rRNA, a gap was detected for the ribose-methylated G at position 2251, whereas this gap was absent from the analysis of unmethylated, 23S rRNA transcribed in vitro (Fig. 2). In the case of yeast mitochondrial 21S rRNA from the *PET56* ρ^+ and *PET56* F11 ρ^- strains, a gap was detected at a position corresponding to ribose-methylated G2270, which is equivalent to Gm2251 in *E. coli* 23S rRNA. A gap was not present at this position in either the 21S rRNA from the *pet56::URA3* F11 ρ^- mutant or the 21S rRNA transcribed in vitro. Densitometric scanning of the autoradiograph in the G2270 region confirmed the similarity between the patterns for the 21S rRNA from the *pet56::URA3* F11 ρ^- mutant and the corresponding unmodified control RNA derived from in vitro transcription (*15*). These data strongly suggest that *PET56* is required for the formation of Gm at a conserved site in the peptidyl transferase center of yeast mitochondrial 21S rRNA.

To test the activity of Pet56p as an rRNA ribose methylase in vitro, we purified the protein by overexpression of *PET56* under the control of the *GAL1* promoter because activity was not detectable in a wild-type yeast strain (*15*). The overproduced Pet56p was localized to mitochondria in an insoluble form resembling bacterial inclusion bodies (*15, 20*), so we followed methods for purifying recombinant proteins from inclusion bodies (*20*). The final preparation of soluble Pet56p was judged to be >90% pure on the basis of Coomassie blue staining after SDS–polyacrylamide gel electrophoresis (*15*) and was used in methylation assays with in vitro–transcribed RNA as substrate and *S*-[*methyl*-^{3}H]adenosyl-L-

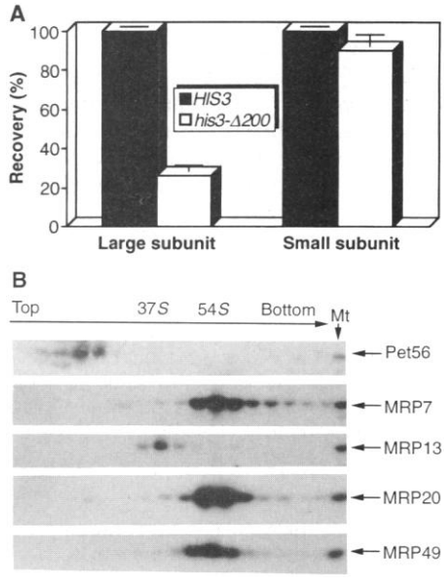

Fig. 1. Relative yield of mitochondrial ribosomal subunits from *HIS3* and *his3-Δ200* strains. (**A**) Mitochondrial lysates were prepared from KSY35 (*HIS3*) and MM1403 (*his3-Δ200*) cells grown on YPGal at 30°C (*12, 24–29*) and analyzed by centrifugation in sucrose gradients containing 500 mM NH_4Cl as in (*14*). The A_{260} (absorbance at 260 nm) profiles of the gradients were monitored with a continuous flow cuvette, and the areas under the peaks for each subunit were normalized to the amount of protein loaded onto each gradient. The average recovery of ribosomal subunits from the *his3-Δ200* mutant is shown as a percentage (± standard deviation) of the recovery for the *HIS3* strain in three experiments. (**B**) The proteins in even-numbered fractions from a sucrose gradient of the *HIS3* mitochondrial lysate were analyzed by immunoblotting with ^{125}I-labeled secondary antibody (*30*). The large subunit was detected with monoclonal antibodies to ribosomal proteins MRP7 (*17*), MRP20, and MRP49 (*14*) and the small subunit with the monoclonal antibody to MRP13 (*30*). The lanes corresponding to the A_{260} peaks for the small (37*S*) and large (54*S*) ribosomal subunits are below the respective labels. A murine polyclonal antibody was used to detect Pet56p (*31*). The lane labeled Mt contains 50 μg of protein from the mitochondrial lysate that was layered onto the gradient.

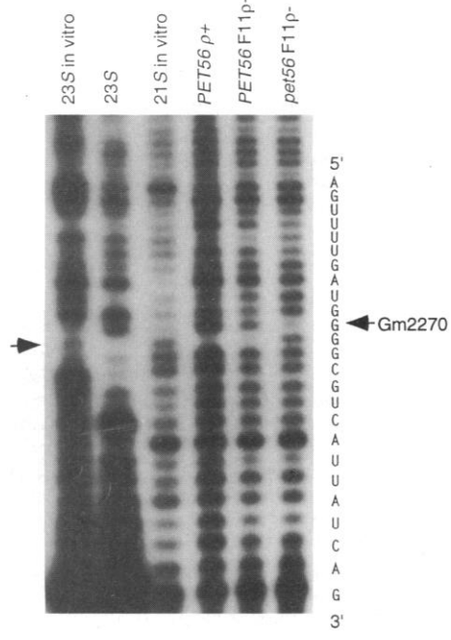

Fig. 2. Primer extension analysis of ribose methylation at G2251 of *E. coli* 23*S* rRNA and the equivalent G2270 yeast mitochondrial 21*S* rRNA. In vitro transcripts containing domains IV and V of either yeast mitochondrial 21*S* rRNA or *E. coli* 23*S* rRNA were generated with T7 RNA polymerase and template pKS10 or pDT1R (*24*). The in vitro transcripts, 23*S* rRNA isolated from *E. coli* ribosomes, and total RNA preparations from the mitochondria (*29*) of COP161 U7 ρ^+ *PET56*, COP161 U7 F11 ρ^- *PET56*, and KSY24 F11 ρ^- *pet56::URA3* cells (*18, 24, 25, 28*) were subjected to partial alkaline hydrolysis by incubation in 50 mM Na_2CO_3 (pH 8.4) for 3 min at 90°C. DNA oligonucleotide primers ^{32}P-labeled at the 5′ end and complementary to nucleotides 2265 to 2286 of the 23*S* rRNA or 2281 to 2305 of the 21*S* rRNA were annealed to the corresponding rRNAs and extended with reverse transcriptase (*32*). The extension products were resolved by electrophoresis in a 6% acrylamide–7 M urea gel. The sequence of the 21*S* rRNA is shown on the right and the gaps corresponding to Gm2251 in 23*S* rRNA and Gm2270 in 21*S* rRNA are indicated by arrows. Although the unmethylated in vitro transcripts for the 21*S* and 23*S* rRNAs and the 21*S* rRNA from the *pet56* mutant gave strong bands instead of gaps at these positions, each of these three samples also consistently gave weak but detectable bands at the next position in the primer extension ladders. The specificity of the primers was confirmed by primer extension of the rRNAs partially digested with base-specific RNases (*15*).

methionine ([^{3}H]SAM) as the methyl donor. The labeled RNA was digested to ribonucleosides, which were then separated by chromatography. The chromatograms were subjected to fluorography, and sections were cut out for the quantitation of radioactivity by liquid scintillation counting. These experiments indicated that Pet56p specifically catalyzes the formation of 2′-*O*-methylguanosine (Fig. 3); no other enzyme-dependent radioactive labeling was detected on the chromatograms.

The Pet56 methylase was substrate-specific, acting on sense transcripts but not antisense transcripts from the domain IV plus V region of yeast mitochondrial 21S rRNA (Fig. 3). In addition, the enzyme was active on sense transcripts from the domain IV plus V region of *E. coli* 23S rRNA, and indeed, the heterologous rRNA substrate consistently resulted in up to 15 times more radioactive Gm than the yeast mitochondrial rRNA substrate. The 23S rRNA isolated from ribosomes was a poor substrate, which is the expected result if ribose methylation at G2251 is essentially complete in mature 23S rRNA (Fig. 2).

The site specificity of the in vitro ribose methylase activity was examined by S1 nuclease protection analysis. The domain IV plus V transcript of 23S rRNA was methylated in a reaction with Pet56p and [^{3}H]SAM and then hybridized to a 100-fold molar excess of deoxyoligonucleotides complementary to either a 28-nucleotide sequence centered on Gm2251 or a 20-nucleotide sequence in domain V that does not contain a modification site. After digestion with S1 nuclease, the protected RNA fragments were precipitated with ethanol and analyzed for ^{3}H-labeled Gm. Only the oligonucleotide complementary to the sequence surrounding G2251 protected the RNA containing ^{3}H-labeled Gm from digestion (Fig. 4). As there is only one naturally occurring ribose methylation site within the protected fragment, and protection was virtually complete (>90%) at the highest concentration of nuclease tested, the Pet56 guanosine methyltransferase appears to act exclusively at position G2251 of *E. coli* 23S rRNA. Furthermore, the fact that this modification reaction was catalyzed by a highly purified enzyme, in the absence of ribosomal proteins, suggests that free rRNA can serve as a substrate, raising the possibility that the modification of G2251 could occur early in the pathway of ribosome assembly.

A large body of information points to an intimate involvement of the large rRNA in the peptidyl transferase reaction of protein synthesis, and specific modified nucleotides could be important structural or functional elements in the catalytic center responsible for this activity. It is relevant, therefore, that the yeast mitochondrial Pet56 ribose methyltransferase is both essential for the formation of functional ribosomes and specific for modification of the universally conserved G corresponding to G2251 in *E. coli* 23S rRNA. Gm2251 occupies a prominent position in the peptidyl transferase center; the adjacent bases, G2252 and G2253, are protected from chemical probing by tRNA bound in the peptidyl (P) site, and C2254 is protected by tRNA in the aminoacyl (A) site (*21*). Because the peptidyl transferase reaction involves the 3′ ends of tRNA, it is noteworthy that oligonucleotide fragments from the 3′ CCA end of acylated tRNA also protect G2252 and G2253 (*21*).

Ribosomal subunits can be reconstituted with rRNAs isolated from ribosomes (*1*). In addition, functional 30S particles have been assembled from 16S rRNA transcribed in vitro (*22*), but so far this has not been accomplished for 50S particles. Moreover, the in vivo assembly of the large subunit but not the small subunit is blocked by ethionine, a methionine analog that supports protein synthesis but not the formation of SAM (*23*). Together, these results suggest that at least one posttranscriptional modification in 23S rRNA is required for the assembly of 50S subunits. Our data point to the formation of 2′-*O*-methylguanosine at G2251 as a necessary step in the synthesis of functional large rRNA; further work will be required to determine whether this modification is sufficient to support large subunit assembly.

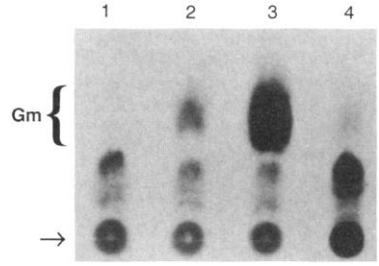

Fig. 3. In vitro methylation by purified Pet56p. Ribose methylase activity was assayed by measuring the transfer of a ^{3}H-labeled methyl group from the methyl donor to the substrate RNAs (*33*). The T7 RNA polymerase and plasmid DNA templates (*25*) were used to generate sense transcripts of domains IV and V of the 21*S* (lane 2) and 23*S* (lane 3) rRNAs and, as a control, an antisense transcript of the 21*S* rRNA (lane 1). In addition, 23*S* rRNA isolated from ribosomes was tested as a substrate (lane 4). The methylation reactions were terminated by extraction with phenol and chloroform and precipitation with ethanol. The RNA was digested to nucleosides and chromatographed on Whatman 3MM paper impregnated with ammonium borate in an aqueous ammonium borate:butanol solvent system. Under these conditions, the four ribose-methylated ribonucleosides can be resolved from each other as well as from base modified nucleosides, which remain near the origin (*34*). The relative mobility of each of the standard ribose-methylated ribonucleosides, visualized by ultraviolet shadowing, were exactly as in (*34*). The chromatogram was sprayed with En^{3}Hance (New England Nuclear) and exposed to x-ray film. Only the region of the fluorogram that contained radioactivity is shown. The absence of radioactive material elsewhere in the chromatogram was confirmed by scintillation counting of 1-cm^2 pieces. Gm indicates the position of 2′-*O*-methylguanosine and the arrow indicates the origin. The radioactive material migrating behind Gm was also present when the ^{3}H-labeled methyl donor alone was chromatographed and when the enzyme was omitted from the reaction mix and is therefore not a product of the enzymatic reaction (*15*). Because this material coprecipitated with the RNA substrate and because the total mass of RNA in the reaction containing the full-length 23*S* rRNA from ribosomes was 2.9 times that of the in vitro transcripts (*24*), the amount of nonspecific trapping of radioactivity was highest in this sample.

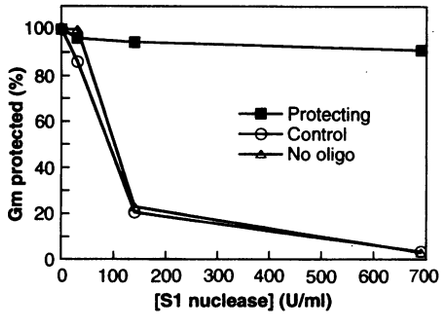

Fig. 4. S1 nuclease protection of the Pet56p methylation site in 23*S* rRNA transcripts. Hybridization reactions in 30 μl of hybridization buffer (*35*) contained 20 μg of 23*S* rRNA methylated in vitro and no deoxyoligonucleotide, a 100-fold molar excess of a 28-nucleotide oligomer complementary to nucleotides 2237 to 2264 (protecting), or a 20-nucleotide oligomer complementary to nts 2608 to 2627 within domain V (control). The reactions were heated at 90°C for 10 min, incubated at 30°C for 1 hour, and then treated with S1 nuclease at concentrations of 0, 28, 138, or 689 U/ml at 37°C for 1 hour (*35*). The RNA was precipitated with ethanol and digested to nucleosides. Nucleosides were separated by chromatography and the amount of radioactive Gm in each sample was determined by liquid scintillation counting. The radioactive Gm recovered is expressed as a percentage of the radioactive Gm in the corresponding samples incubated in the absence of S1 nuclease [100% = ~13,000 cpm in each of the three hybridization reactions].

REFERENCES AND NOTES

1. H. F. Noller, *Annu. Rev. Biochem.* **60**, 191 (1991).
2. _____, V. Hoffarth, L. Zimniak, *Science* **256**, 1416 (1992).
3. R. Brimacombe, P. Mitchell, M. Osswald, K. Stade, D. Bochkariov, *FASEB J.* **7**, 161 (1993).
4. J. Nichols and B. Lane, *J. Mol. Biol.* **30**, 477 (1967); C. Branlant *et al.*, *Nucleic Acids Res.* **9**,

4303 (1981); J. E. Smith, B. S. Cooperman, P. Mitchell, *Biochemistry* **31**, 10825 (1992).
5. D. Dubin and R. Taylor, *J. Mol. Biol.* **121**, 523 (1978); R. Baer and D. Dubin, *Nucleic Acids Res.* **9**, 323 (1981).
6. J. Klootwijk, I. Klein, L. Grivell, *J. Mol. Biol.* **97**, 337 (1975); A. Lambowitz and D. Luck, *J. Biol. Chem.* **251**, 3081 (1976).
7. K. Struhl, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8419 (1985).
8. ———, *Nucleic Acids Res.* **13**, 8587 (1985).
9. K. Sirum-Connolly and T. L. Mason, GenBank accession number L19947.
10. GenBank accession numbers X02392 and M32744; J. Devereux, P. Haeberli, O. Smithies, *Nucleic Acids Res.* **12**, 387 (1984); S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990); Pet56p also has 64% similarity to a partial sequence (138 amino acids) of the Pet56p homolog in *Saccharomyces kluyveri* (GenBank accession number Z14125).
11. E. Cundliffe and J. Thompson, *J. Gen. Microbiol.* **126**, 185 (1981); J. Thompson and E. Cundliffe, *Biochimie* **73**, 1131 (1991).
12. Rich medium was composed of 1% yeast extract, 2% bacto-peptone, and either 2% galactose (YP-Gal), or 2% glycerol and 2% ethanol (YPGE).
13. K. Sirum-Connolly, K. Fearon, T. L. Mason, unpublished results.
14. K. Fearon and T. L. Mason, *J. Biol. Chem.* **267**, 5162 (1992).
15. K. Sirum-Connolly and T. L. Mason, unpublished results.
16. A. M. Myers, L. K. Pape, A. Tzagoloff, *EMBO J.* **4**, 2087 (1985).
17. K. Fearon and T. L. Mason, *Mol. Cell. Biol.* **8**, 3636 (1988).
18. G. Faye, C. Kujawa, H. Fukuhara, *J. Mol. Biol.* **88**, 185 (1974); G. Faye, *FEBS Lett.* **69**, 167 (1976); V. S. Parikh, M. M. Morgan, R. Scott, L. S. Clements, R. A. Butow, *Science* **235**, 576 (1987); S. Merten, R. M. Synenki, J. Locker, T. Christianson, M. Rabinowitz, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1417 (1980).
19. B. Maden and M. Salim, *J. Mol. Biol.* **88**, 133 (1974).
20. F. A. O. Marston, *Biochem. J.* **240**, 1 (1986); A. Hoess, A. K. Arthur, G. Wanner, E. Fanning, *Bio/Technol.* **6**, 1214 (1988).
21. D. Moazed and H. F. Noller, *Cell* **57**, 585 (1989); *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3725 (1991).
22. W. Krzyzosiak *et al.*, *Biochemistry* **26**, 2353 (1987); P. Melançon, M. Gravel, G. Boileau, L. Brakier-Gingras, *Biochem. Cell Biol.* **65**, 1022 (1987); P. Cunningham, R. Richard, C. Weitzmann, K. Nurse, J. Ofengand, *Biochimie* **73**, 789 (1991).
23. M. Chelbi-Alix, A. Expert-Benzancon, F. Hayes, *Eur. J. Biochem.* **115**, 627 (1981).
24. Plasmids were constructed as follows. We obtained pKS1 and pKF513 by ligating a 2.8-kb Pst I fragment containing *PET56* from Ylp5-Sc3339 into the Pst I site of M13mp19 and pUC18, respectively. The plasmid pKS3 was derived from pKF513 by removal of a 710-bp Nco I–Apa I fragment within the coding region of the *PET56* gene and insertion of the *URA3* gene flanked by *hisG* repeats from the plasmid pNKY51 (*25*). The plasmid pDT1R contains sequences encoding domains IV and V of the 23*S* rRNA under the control of a T7 promoter. We obtained pKS9 and pKS10 by inserting, in both orientations, a 985-bp Ava II fragment of yeast mitochondrial 21*S* rDNA from an intronless *omega*$^-$ strain at the Sma I site of pBluescript KS(+) (Stratagene) such that the T7 promoter could be used to generate sense (pKS10) and antisense (pKS9) transcripts of domain IV plus V of 21*S* rRNA. We created pKS13, a multicopy 2-μm based vector containing a *GAL1-PET56* promoter fusion, by fusing *PET56* at −15 with respect to the ATG start codon to the *GAL1* promoter in pB656 (*26*). And lastly, pKF517 was obtained by ligation of a 1.8-kb Bam HI–Pst I fragment from pKF513 into pATH10 (*27*) and was used to express a TrpE-Pet56p fusion protein in *E. coli*.
25. E. Alani, L. Cao, N. Kleckner, *Genetics* **116**, 541 (1987).
26. M. I. Ciu, T. L. Mason, G. R. Fink, *ibid.* **132**, 987 (1992).
27. T. J. Koerner, J. E. Hill, A. M. Myers, A. Tzagoloff, *Methods Enzymol.* **194**, 477 (1991).
28. *Saccharomyces cerevisiae* transformations and genetic analyses were done by standard methods. The following yeast strains were used: MM1403 ρ$^+$ (*MAT***a** *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ101 ura3-52*); COP161 U7 ρ$^+$ (*MATα ade lys ura3*) and the isonuclear F11ρ$^-$ derivative (*18*); the *HIS3* strain, KSY35 ρ$^+$ (*MAT***a** *ade2-101 leu2-Δ1 lys2-801 trp1-Δ101 ura3-52*) [obtained from MM1403 by transformation with a Pst I fragment from pKS1 (*24*)]; and KSY24 F11ρ$^-$ (*MATα ade lys pet56::URA3*) [generated from COP161 U7 F11ρ$^-$ by transformation with a Eco RI–Sph I fragment from pKS3 (*18, 24*)].
29. G. Daum, P. C. Bohni, G. Schatz, *J. Biol. Chem.* **257**, 13028 (1982); H. Zhu, I. G. Macreadie, R. A. Butow, *Mol. Cell. Biol.* **7**, 2530 (1987).
30. J. A. Partaledis and T. L. Mason, *Mol. Cell. Biol.* **8**, 3647 (1988).
31. The Pet56 polyclonal antibody was hyperimmune ascites fluid from mice immunized with a recombinant TrpE-Pet56p fusion protein expressed in *E. coli* from pKF517 (*24, 27*).
32. E. Kuechler, G. Steiner, A. Barta, *Methods Enzymol.* **164**, 361 (1988).
33. The 50-μl methylation reaction mixture contained ~50 μg of purified Pet56 enzyme, 1 μM RNA, 2 μM [^{3}H]SAM (80 Ci/mmol, New England Nuclear), 50 mM tris-HCl (pH 8.0), 50 mM KCl, 2 mM dithiothreitol, 4 mM EDTA, and bovine serum albumin (0.4 mg/ml), and the reactions were incubated at 30°C for 3 hours; D. Eichler, N. Raber, C. Shumard, S. Eales, *Biochemistry* **26**, 1639 (1987).
34. A. Al-Arif and M. Sporn, *Anal. Biochem.* **48**, 386 (1972).
35. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1989).
36. We thank D. Thurlow and K. Struhl for providing plasmids; C. Martin for T7 RNA polymerase; J. Woolford for the strain MM1403; J. Wower, K. Rosen, R. Zimmermann, and T. Fox for advice and discussions; and K. Fearon for help in the early stages of the work. Supported by NSF grant DMB-8719558 to T.L.M.

21 June 1993; accepted 29 October 1993

Inhibition of Transcriptional Regulator Yin-Yang–1 by Association with c-Myc

A. Shrivastava, S. Saleque, G. V. Kalpana, S. Artandi, S. P. Goff, K. Calame*

Yin-Yang–1 (YY1) regulates the transcription of many genes, including the oncogenes c-*fos* and c-*myc*. Depending on the context, YY1 acts as a transcriptional repressor, a transcriptional activator, or a transcriptional initiator. The yeast two-hybrid system was used to screen a human complementary DNA (cDNA) library for proteins that associate with YY1, and a c-*myc* cDNA was isolated. Affinity chromatography confirmed that YY1 associates with c-Myc but not with Max. In cotransfections, c-Myc inhibits both the repressor and the activator functions of YY1, which suggests that one way c-Myc acts is by modulating the activity of YY1.

Zinc finger protein Yin-Yang–1 (CF1, δ, NF-E1, or UCRBP) is a 65-kD DNA binding protein, belonging to the GLI-Krüppel family, which is widely expressed and highly conserved between humans and mice (*1*). Depending on the context, YY1 can function as an activator, a repressor, or an initiator of transcription (*1*). YY1 represses the adeno-associated virus (AAV) P5 promoter (*1*), the immunoglobulin (Ig) κ 3′ enhancer (*1*), the c-*fos* promoter (*2*), the human papilloma virus–18 promoter (*3*), the skeletal α-actin promoter (*4*), the long terminal repeat of Moloney murine leukemia virus (*1*), and on the basis of sequence similarity, the N-*ras* promoter (*3, 5*). YY1 repression of the P5 promoter is relieved by adenovirus E1A protein (*1*). In some of these genes YY1 DNA binding sites overlap with serum response elements or 12-O-tetradecanoyl phorbol-13-acetate (TPA) response elements and repression is relieved when activators such as serum response factor or AP-1 displace YY1 (*2–4, 6*). A fusion protein of YY1 and the GAL4 DNA binding domain represses transcription of a thymidine kinase promoter with GAL4 binding sites (*1*). However, YY1 is an activator for the c-*myc* promoter, the IgH intronic enhancer, and the promoters of ribosomal proteins L30 and L32 (*7*). Finally, YY1 acts as a transcriptional initiator at nucleotide position +1 in the AAV P5 promoter (*8*).

A simple model to explain the functional versatility of YY1 is that interactions

A. Shrivastava, S. Saleque, G. V. Kalpana, Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032.
S. Artandi, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY 10032.
S. P. Goff, Department of Biochemistry and Molecular Biophysics, Department of Microbiology, and Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, New York, NY 10032.
K. Calame, Department of Biochemistry and Molecular Biophysics and Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY 10032.
*To whom correspondence should be addressed.