5 S rRNA Is Involved in Fidelity of Translational Reading Frame

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Manuscript received January 20, 1995
Accepted for publication June 9, 1995

ABSTRACT

Chromosomal mutants (maintenance of frame = *mof*) in which the efficiency of −1 ribosomal frameshifting is increased can be isolated using constructs in which *lacZ* expression is dependent upon a −1 shift of reading frame. We isolate a new *mof* mutation, *mof9*, in *Saccharomyces cerevisiae* and show that it is complemented by both single and multi-copy 5 S rDNA clones. Two independent insertion mutations in the rDNA locus (*rDNA::LEU2* and *rDNA::URA3*) also display the Mof$^-$ phenotype and are also complemented by single and multi-copy 5 S rDNA clones. Mutant 5 S rRNAs expressed from a plasmid as 20–50% of total 5 S rRNA in a wild-type host also induced the Mof$^-$ phenotype. The increase in frameshifting is greatest when the *lacZ* reporter gene is expressed on a high copy, episomal vector. No differences were found in 5 S rRNA copy number or electrophoretic mobilities in *mof9* strains. Both *mof9* and *rDNA::LEU2* increase the efficiency of +1 frameshifting as well but have no effect on readthrough of UAG or UAA termination codons, indicating that not all translational specificity is affected. These data suggest a role for 5 S rRNA in the maintenance of frame in translation.

ALTHOUGH correct maintenance of frame is critical to ribosome function, a growing number of cases of directed alterations in translational reading frame have been identified, mostly in viruses (*e.g.*, retroviruses, coronaviruses, the L-A dsRNA virus of *Saccharomyces cerevisiae*, (+) ssRNA plant viruses and bacteriophage T7), as well as in some yeast retrotransposable elements, bacterial transposons and a few bacterial genes (reviewed in JACKS 1990; ATKINS *et al.* 1991; HATFIELD *et al.* 1992; CHANDLER and FAYET 1993; FARABAUGH 1993). The study of these ribosomal frameshifts is important both because of their critical role in animal and plant pathogens and because of the information they provide about the mechanisms by which reading frame is normally maintained.

Ribosomal frameshifting in the −1 direction in retroviruses, (+) ssRNA viruses and dsRNA viruses generally requires a special sequence, X XXY YYZ (the 0-frame is indicated by spaces) called the "slippery site" (JACKS *et al.* 1988). Here the simultaneous slippage of ribosome-bound A- and P-site tRNAs by one base in the 5′ direction still leaves their nonwobble bases correctly paired in the new reading frame. A second promoting element (JACKS *et al.* 1988), usually an RNA pseudoknot, is located immediately 3′ to the slippery site (BRIERLEY *et al.* 1989, TEN DAM *et al.* 1992). The RNA pseudoknot makes the ribosome pause over the slippery site (TU *et al.* 1992, SOMOGYI *et al.* 1993), increasing the probability of 5′ ribosomal movement. The efficiency of −1 ribosomal frameshifting can be affected by the ability of the ribosome-bound tRNAs (especially the A-site tRNA) to un-pair from the 0-frame (DINMAN *et al.* 1991, BRIERLEY *et al.* 1992), the ability of these tRNAs to re-pair to the −1 frame (JACKS *et al.* 1988), and the relative position of the RNA pseudoknot from the slippery site and its thermodynamic stability (BRIERLEY *et al.* 1989, 1991; DINMAN and WICKNER 1992). The efficiency of −1 ribosomal frameshifting can also be affected by mutations in cellular gene products that presumably interact with these tRNA and mRNA factors (DINMAN and WICKNER 1992, 1994). Changes in any of these components can be observed as changes in the efficiency of −1 ribosomal frameshifting, and their analysis promises to shed light upon the mechanisms underlying the maintenance of frame in translation.

The 4.6-kb dsRNA L-A virus of *S. cerevisiae* has two open reading frames. The 5′ *gag* encodes the major coat protein and the 3′ *pol* gene encodes a multifunctional protein domain that includes the RNA-dependent RNA polymerase and a domain required for viral RNA packaging (HOPPER *et al.* 1977, ICHO and WICKNER 1989, FUJIMURA *et al.* 1992, RIBAS and WICKNER 1992). A −1 ribosomal frameshift event is responsible for the production of the Gag-Pol fusion protein (FUJIMURA and WICKNER 1988, ICHO and WICKNER 1989, DINMAN *et al.* 1991). The satellite dsRNA virus of L-A, M_1, which encodes a secreted killer toxin (reviewed by BUSSEY 1991), is encapsidated and replicated in L-A encoded proteins. The efficiency of the −1 ribosomal frameshift event is critical for M_1 maintenance (DINMAN and WICKNER 1992, 1994). Changing frameshifting efficiencies greater than two- to threefold by altering the slippery sites in L-A cDNA clones, or as a result of mutations in host en-

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TABLE 1

Strains and crosses of *S. cerevisiae*

Strains	Genotypes or parents
2907	*MAT***a** *his3-δ200 leu2⁻ trp1-δ901 ura 3-52 ade2-10* K⁻
EMS56	*MAT***a** *his3-δ200 leu2⁻ trp1-δ901 ura 3-52 ade2-10 mof9-1* K⁻ + p-1
1995	*MATα spo11 ura2 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1* K⁺
Cross JD18	EMS56 × 1995
2908	*MATα his3-δ200 leu2⁻ trp1-δ901 ura3-52 ade5* K⁻
AH22(929)	*MAT***a** *leu2⁻ his4 can1 gal1 rDNA::LEU2* (From T. PETES)
Cross 4377	2908 × AH22 (929)
TP406pSS31	*MAT***a** *leu2 ura3 lys11 gal2 his5 rDNA::URA3* (From T. PETES)
Cross 4378	2908 × TP406pSS31
4377-8C	*MATα trp1 his3 his4 ura3 leu2 rDNA::LEU2*
Cross 745	EMS56 × 4377-8C
Cross 746	EMS56 (+p0) × 4377-8C
JD112	*MATα ura3-52 trp1-δ901* K⁺
4378-5B	*MATα leu2⁻ lys11 trp1-δ901 ura3-52* K⁻
1074	*Mat***a** *leu1 kar1-1* L-AHN M_1
2631	*Mat***a** *leu1 kar1-1* L-AHNB M_1
3166	*MATα leu1 kar1-1 arg1 thr(1,x)* L-AHN M_1
JD18-3C	*MATα ura3-52 ade2-10 ade6 met14 trp-1-δ901 mof9-1* K⁻
4377-8B	*MAT***a** *trp1 leu2⁻ his3 his4* K⁻

coded genes, results in the loss of the M_1 satellite dsRNA. Similarly, increasing or decreasing the efficiency of +1 ribosomal frameshifting in the yeast retrotransposable element Ty*1* decreases its transposition efficiency (XU and BOEKE 1990, KAWAKAMI *et al.* 1993, BALASUNDARAM *et al.* 1994). These findings support the hypothesis that agents that affect ribosomal frameshifting efficiency may have anitviral activities.

Using −1 ribosomal frameshifting as an assay, we have reported the preliminary characterization of eight genes of *S. cerevisiae* that are involved in the maintenance of frame (*MOF*) in translation. All of these increase the efficiency of −1 ribosomal frameshifting by the L-A frameshifting signal. Here we describe a new Mof⁻ mutant, *mof9*, whose analysis implicates 5 S rRNA in the maintenance of reading frame.

MATERIALS AND METHODS

Strains and media: The strains of *S. cerevisiae* used are listed in Table 1. YPAD, YPG, SD, and synthetic complete medium (H-trp, −ura) were as previously described (SHERMAN 1991). To synthetic complete medium was added 100 mM $NaPO_4$ pH 6.8 and 40 μg/ml 5-bromo-4-chloroindolyl-β-D-galactopyranoside (X-gal) for screening β-galactosidase production. *Escherichia coli* strains DH10 (Bethesda Research Laboratories) and JM109 were used for production of plasmid DNA.

Genetic methods: Transformation of yeast was by the lithium acetate method (ITO *et al.* 1983), and transformation of *E. coli* was by the calcium chloride method (MANIATIS *et al.* 1982). Genetic crosses, sporulation and tetrad analyses were performed as described previously (SHERMAN 1991, WICKNER 1991) with the modification that to select for episomal plasmids, crosses were germinated on H-trp agar. The *mof9* mutant EMS56 was generated by ethyl methanesulfonate (EMS) (LAWRENCE 1991) and was identified in the same screen that yielded *mof1* (called *hsh1* in DINMAN and WICKNER 1992).

Plasmids: Plasmids pTI25 and pF8 have been described (DINMAN *et al.* 1991). Briefly, pTI25 is the 0-frame control plasmid, while pF8 is the −1 ribosomal frameshift tester. Here we refer to them as p0 and p−1, respectively.

p−1 was mutagenized with oligonucleotide JD115a (5′ GTACTCAGCAGGGT **TAGA** GGAGTGGTAGGTC 3′) and JD115o (5′ GTACTCAGCAGGGT **TAAA** GGAGTGGTAGGTC 3′), placing the *lacZ* reporter gene in the 0 frame with amber and ochre codons, respectively, blocking its translation. YCp50 (JOHNSTON and DAVIS 1984), pRS316 (SIKORSKI and HIETER 1989) and pRS426 (CHRISTIANSON *et al.* 1992) were used for cloning and subcloning. pFS22 is a YCp50 based clone (from ATCC yeast genomic library, CEN BANK #37415) (ROSE *et al.* 1987) that was found to complement the *mof9* mutation. pJD64 is pRS316 containing the 2.1-kb. *Eco*RI B fragment (PETES *et al.* 1978) of pFS22 and pJD106 is the same *Eco*RI fragment in pRS426. pYF404 (vector only), pYF404Y5 (vector containing a 5 S rDNA clone), pYF404Y5G98 (5 S rDNA clone with C98 → G mutation) and pYF404Y5A99 (5 S rDNA clone with G99 → A mutation) (VAN RYK *et al.* 1990) were kindly provided by R. NAZAR. The 436-bp *Bam*HI fragments from pYF404Y5, pYF404Y5G98, and pYF404Y5A99 were subcloned into pEMBLyex4 (BALDARI *et al.* 1987) to construct pJD116Y5, pJD116Y5C98G and pJD116Y5G99A, respectively. Cells containing p0 or p−1 were transformed with these plasmids and grown on H-trp-ura medium. Individual colonies from these transformations were then grown in H-trp-leu medium to amplify the pJD116Y5 series plasmids.

β-galactosidase assays: Assays of permeabilized yeast cells were as described previously (GUARENTE 1983). Cells were grown in H-trp, H-trp-ura, or H-trp-leu medium to the midlogarithmic phase, and assays were normalized with respect to the optical density at 595 nm of the culture and to the assay time. All assays were performed in triplicate. β-galactosidase activities are expressed as the change in $A_{420}/hr/OD_{595}$. Percent frameshifting is derived from comparison of β-galactosidase activities of cells containing p−1 *vs.* p0.

Preparation of DNA: Extraction of genomic DNA was by the yeast spheroplast method described previously (DINMAN and WICKNER 1994). Samples were resuspended in 10 mM Tris·Cl pH 8.0, 1 mM EDTA (TE) and 1 μg/sample was di-

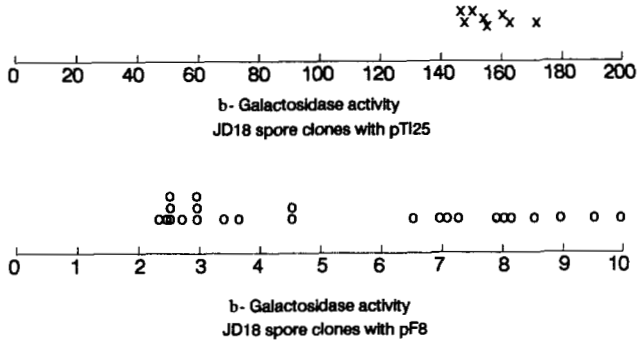

FIGURE 1.—Bimodal distribution of −1 ribosomal frameshifting in *mof9* mutants. Six complete tetrads (24 spore clones) from cross JD18 containing p−1 were assayed for β-galactosidase activities as described in MATERIALS AND METHODS (bottom). Subsequently, eight spore clones from two complete tetrads were cured of p−1, retransformed with p0, and assayed for β-galactosidase activities (top).

gested with *Eco*RI, *Hae*III, *Hin*dIII or *Sal*I (BRL) overnight at 37°. Samples were separated through a 1% Tris-acetate-EDTA agarose gel containing 0.5 μg of ethidium bromide/ml and transferred to Nylon (Hybond-N, Amersham) as recommended by the manufacturer.

Plasmid DNA was recovered from yeast cells by lysis with glass beads in 100 mM NaCl, 10 mM Tris·Cl pH 8.0, 1 mM EDTA, 1% SDS, 2% Triton X-100. For slot blot analysis, phenol, phenol/chloroform and chloroform extractions were performed and DNA was precipitated with ethanol. The DNA was suspended in 200 μl of TE, and 200 μl of 7.5 M ammonium acetate was added. After incubation on ice for 20 min and centrifugation, the supernate was collected and incubated with RNase A for 2 hr at 37°. The solution was again incubated on ice for 20 min and, following centrifugation, the supernatant was collected. DNA was precipitated with ethanol and resuspended in TE. OD_{260}/OD_{280} was determined and samples were diluted to 0.1 mg/ml. Two micrograms of sample was diluted to 70 μl in TE. Plasmid was denatured at room temperature by incubation with 20 μl of 2 N NaOH for 5 min, 30 μl of ice cold 3 M sodium acetate pH 4.5 was added, and the samples were placed on ice. An additional 80 μl of 2× SSC was added to a final volume of 200 μl (0.01 μg/μl). One hundred microliters (1 μg) was initially applied onto a nylon (Hybond-N) membrane assembled in a slot blot apparatus (BRL). Samples were serially diluted with 100 μl 2× SSC and sequentially applied to the membrane. The membrane was washed with 2× SSC and the DNA was fixed by baking in a vacuum oven at 80° for 1 hr.

Preparation of total nucleic acids: Equal OD_{550} of cells were extracted with glass beads as described above. Total nucleic acids were precipitated with ethanol and were separated on an 8% acrylamide Tris-borate-EDTA urea gel and stained with 0.5 μg/ml ethidium bromide. 5 S RNA bands were excised from the gel, extracted with TE with 0.3 M sodium acetate pH 5.2, 0.1% SDS, 5000 U/ml RNasin and precipitated with ethanol. Gel-purified 5 S rRNA was dephosphorylated with bacterial alkaline phophatase (BRL) in 10 mM Tris Cl, pH 8.0, with 5000 U/ml RNasin, extracted twice each with phenol, phenol-chloroform and chloroform and ethanol precipitated. 5 S rRNA was resuspended in 100 mM Tris Cl pH 8.0, 10 mM $MgCl_2$, 5 mM dithiothreitol and 5000 U/ml RNasin and was 5′ end labeled with ^{32}P-ATP and polynucleotide kinase (New England Biolabs). End-labeled 5 S rRNA (2×10^3 cpm) was separated through a 40-cm 12% polyacrylamide Tris-borate EDTA gel (nondenaturing conditions) at 1000 V for 7 hr. The gel was dried and exposed for autoradiography.

TABLE 2

Meiotic segregation of *mof9*

	Spore			
Tetrad	A	B	C	D
JD18-1 p-1	9.6[a]	3.0	2.5	6.6
JD18-2 p-1	7.0	2.5	2.3	4.6
JD18-3 p-1	3.7	3.0	10.0	9.0
JD18-4 p-1	8.7	2.5	4.6	7.9
JD18-5 p-1	7.3	2.7	8.1	3.0
JD18-6 p-1	2.6	8.3	7.2	3.4
JD18-1 p0[b]	158	146	172	161
JD18-3 p0	149	145	162	157

[a] β-galactosidase activities expressed from p-1 or p0, as indicated. Original spore clones from cross JD18 all contained p-1. Six complete tetrads were assayed in triplicate.

[b] Two tetrads of cross JD18 were cured of p-1 and re-transformed with p0. Two transformants of each spore clone were assayed in triplicate.

***In vivo* labeling of RNA:** Yeast were grown to the midlogarithmic phase in YPAD depleted of phosphate (RUBIN 1973). Cells (~3×10^7) were resuspended in 1 ml of phosphate-depleted YPAD containing 63 μCi of carrier free [^{32}P]-orthophosphate (ICN) and grown at 30° for 1 hr. Total RNA was harvested as described above and separated on 8% denaturing gels and 8% nondenaturing gels. Gels were exposed for autoradiography and 5 S and 5.8 S RNA bands were quantitated by optical densitometry.

Hybridizations: For southern blots, pJD64 was digested with *Eco*RI, and the products separated by electrophoresis on a 0.8% agarose gel, the 2.1-kb fragment was eluted and labeled by random primer extension with [α-^{32}P]dCTP as previously described (FEINBERG and VOGELSTEIN 1983). The probe for the slot blot was prepared by digestion of pBR322 with *Eco*RI and also labeled by random primer extension. DNA-DNA hybridizations were as previously described (CHURCH and GILBERT 1984). RNA-RNA hybridizations were carried out as previously described (DINMAN and WICKNER 1994). 5 S rRNA (−) strand probe was made by T7 RNA polymerase run-off transcription of *Cla*I-digested pJD64. 5 S rRNA (+) strand probes were run-off transcripts made with T3 polymerase using *Bam*HI-cleaved pJD64. LacZ (−) strand probe was made by T7 RNA polymerase run-off transcription of *Xho*I-digested pJD86 (DINMAN and WICKNER 1994).

Preparation of exonuclease III deletion mutants: Exonuclease III deletion and ligation of pJD64 and transformation made use of the Erase-A-Base kit (Promega). For the *Hin*dIII-end (left end) deletions, pJD64 was digested with *Kpn*I and *Hin*dIII; for the *Xba*I-end (right end) deletions pJD64 was digested with *Xba*I and *Sac*I. The sequence of the pJD64 deletion mutants was determined using modified T7 DNA polymerase (TABOR and RICHARDSON 1987) (Sequenase V.2.0, United States Biochemical) using standard −20 and reverse primers (United States Biochemical). Sequence analysis used the Geninfo service of NCBI.

RESULTS

Isolation of *mof9*: Strain 2907 cells containing the frameshift detection plasmid p−1 (previously refered to as pF8) (DINMAN *et al.* 1991, DINMAN and WICKNER

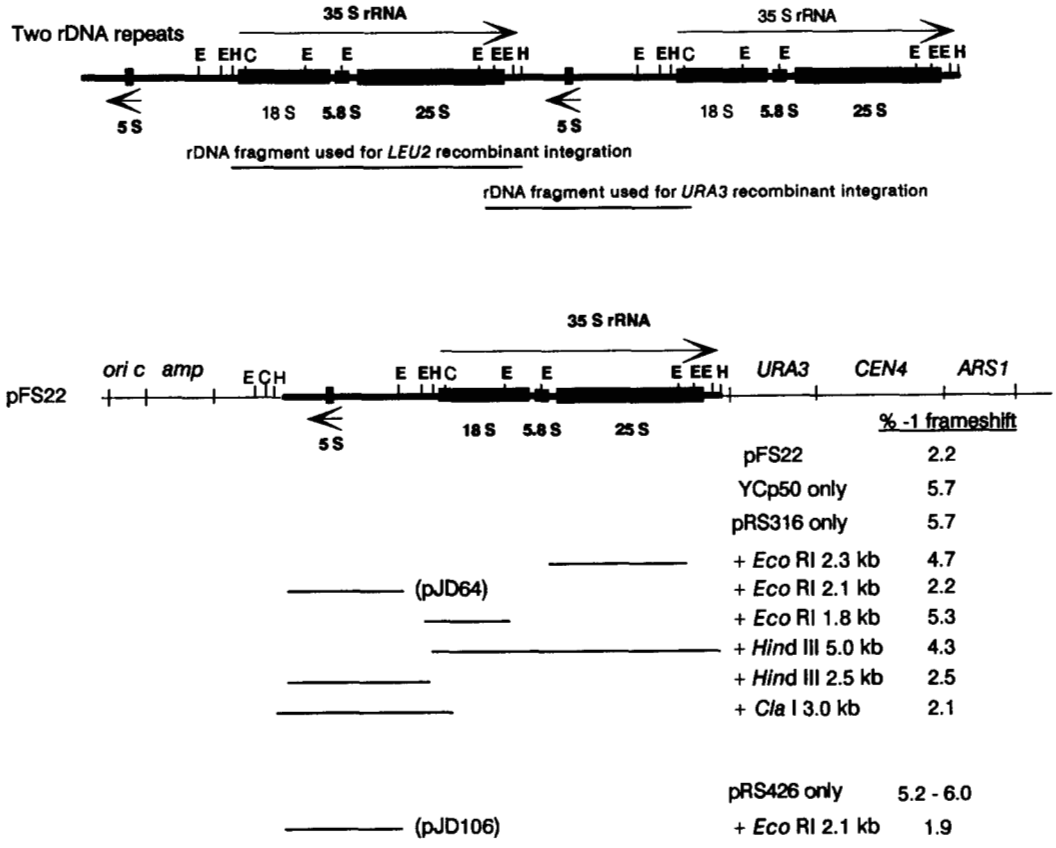

FIGURE 2.—Restriction maps of the rDNA repeat and plasmids used to complement the *mof9*, *rDNA::LEU2* and *rDNA::URA3* integrative recombinant mutations. (Top) Two tandem rDNA repeats. Approximate locations of fragments used to construct the *rDNA::LEU2* and *rDNA::URA3* integrative recombinants are shown. (Middle) Map of pFS22, one complete rDNA repeat cloned into YCp50. (Bottom) Restriction fragments cloned into pRS316 and pRS426 and their abilities to complement the *mof9* mutation as expressed by percentage −1 ribosomal frameshifting efficiencies. 35 S and 5 S rRNA transcripts are denoted by arrows. The regions corresponding to mature 18 S, 5.8 S, 5 S and 25 S rRNAs are noted. Restriction sites: E, *Eco*RI; H, *Hin*dIII; C, *Cla*I.

1992, 1994) were mutagenized with EMS and spread on H-trp X-gal plates. The *mof9* mutation was identified by the dark blue phenotype of a single colony (EMS56). EMS56 was cured of p−1 and was retransformed with p−1 or p0 (previously refered to as pTI25) (DINMAN *et al.* 1991, DINMAN and WICKNER 1992, 1994). From β-galactosidase activities with each plasmid the efficiency of −1 ribosomal frameshifting was 5.7% in EMS56 and 1.9% in unmutagenized cells, a ratio of 3.0. In a meiotic cross with a wild-type strain (EMS56 × 1995 = cross JD18) β-galactosidase activity showed a bimodal distribution with 11 high, *i.e.*, *mof9* (mean ± σ β-galactosidase activity = 8.2 ± 1.1) and 13 low, *i.e.*, MOF^+ (3.1 ± 0.7) segregants (Figure 1). Five of the six tetrads showed 2 high:2 low segregation. The ratio of mutant/wild-type was 2.6. Two complete tetrads (eight spore clones) from cross JD18 were cured of p−1, retransformed with p0 and were assayed for their β-galactosidase activities. There were no significant differences in 0 frame β-galactosidase activities between *mof 9* (159 ± 5.0) and MOF^+ (151.3 ± 11.9) segregants transformed with p0 (Table 2). Frameshifting efficiencies were 5.1% in *mof9* and 2.0% in MOF^+. In addition we observed that the dark blue colonies on X-gal plates were flocculent in liquid medium.

Cloning of *mof9* by complementation: JD18-3C (*mof9*) carrying p−1 was transformed with a YCp50 based yeast genomic library, and 4000 transformants were replica plated to H-trp-ura + X-gal. Three light blue colonies were identified and were assayed in liquid medium. The β-galactosidase activity of one of these, isolate 22, had a frameshifting efficiency of 2.2%. The plasmid (pFS22) was extracted from the original isolate and retransformed into EMS56 and JD18-3C, each carrying p−1 or p0. Cells transformed with this plasmid were no longer flocculent in liquid medium and the efficiency of −1 ribosomal frameshifting was 2.2%. pFS22 did not complement the elevated frameshifting of mutants in *mof1* through *mof8* supporting the designation of this as a new *MOF* locus. The *mof9* strains JD18-3C and EMS56 transformed with the vector, YCp50, showed 5.7 and 4.7% frameshifting, respectively, and were flocculent in liquid culture (Figure 2). The flocculence of *mof9* cells did not affect the measurement of β-galactosidase activity as shown by the lack of any effect on β-galactosidase from the 0 frame control plasmid.

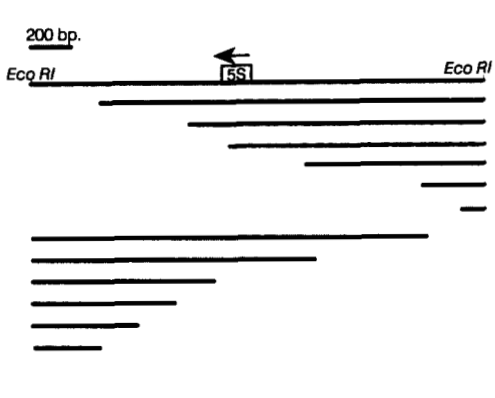

FIGURE 3.—The 5 S rRNA gene complements *mof9*–1. Deletion derivatives of pJD64 were constructed, using exonuclease III, removing either part of the left end (L series) or the right end (R series) of the insert (see Figure 2). The approximate extent of deletion determined by restriction digestion is indicated in bp following the Δ. JD18-3C cells were transformed with p−1 or p0 and subsequently retransformed with plasmids from the pJD64 series of plasmids. β-galactosidase activities and percentage −1 ribosomal frameshifting efficiencies were calculated as described in MATERIALS AND METHODS.

The overlapping 2.1-kb *Eco*RI, 2.5-kb *Hin*dIII and 3.0-kb *Cla*I fragments of pFS22 in the vector pRS316 were able to complement the *mof9* mutation (Figure 2). All of the other subclones and pRS316 alone were unable to complement the *mof9* mutation (Figure 2). The 2.1-kb *Eco*RI fragment cloned into pRS316 was designated pJD64.

A nested set of deletions from the right end (as oriented in Figure 2) and from the left end of the pJD64 insert were made with exonuclease III and were transformed into *mof9* cells. The complementing region begins within 300–680 bp from the right side and 340–780 bp from the left side of the 2.1-kb *Eco*RI insert (Figure 3). Sequence analysis determined a match with the *Eco*RI B fragment of the rDNA repeat that includes the 5 S rRNA gene and the nontranscribed spacer region (JEMTLAND *et al.* 1986). Our restriction map of pFS22 is identical to published maps of the yeast ribosomal RNA gene (MCMAHON *et al.* 1984; JEMTLAND *et al.* 1986) (Figure 2).

Analysis of rDNA::LEU2 and rDNA::URA3 integrative recombinant mutants: Complementation of *mof9*–1 by the 5 S rRNA gene suggested that *mof9–1* might be a mutation of the rDNA. To test the linkage of *mof9–1* to the rDNA repeat, we obtained an rDNA::LEU2 strain from T. PETES, constructed by integrating into the rDNA locus plasmid pTP9, that has the large (5.0 kb) *Hin*dIII fragment of form II rDNA (lacking 5 S sequences) in the *LEU2* vector CV9 (PETES 1980). pSS31 contains the part of rDNA shown in Figure 2 (including the 5 S gene) inserted by dA-dT tailing into the *Eco*RI site of pMB9. It was integrated by homology into rDNA to produce rDNA::URA3 strains (PETES 1980). To facilitate the introduction of frameshift indicator plasmids, the rDNA::LEU2 and rDNA::URA3 strains were mated to wild-type strains to introduce *trp1* (crosses 4377 and 4378). *trp1* spore clones from each cross were transformed with p−1 and p0 and were assayed for β-galactosidase activities (Table 3a). The rDNA::LEU2 and rDNA::URA3 strains had an average −1 ribosomal frameshifting efficiency of 8.5 and 8.9%, respectively, and both were flocculent. Wild-type spore clones frameshifted with 1.8% efficiencies. Diploids of genotype rDNA::URA3/*mof9* had an average −1 ribosomal frameshifting efficiency of 8.7% whereas rDNA::LEU2/rDNA (wt) diploids and *mof9*/MOF (wt) diploids showed average efficiencies of 2.0 and 2.4%, respectively (Table 3b).

rDNA::LEU2/*mof9* diploids containing p−1 (cross JD745) or p0 (cross JD746) were sporulated and subjected to tetrad analysis (Table 3c). In cross JD745, no significant differences were observed in β-galactosidase activities of 24 spore clones representing six tetrads. The β-galactosidase activities were uniformly high [10.2 ± 1.7 for Leu$^+$ (rDNA::LEU2) segregants and 9.6 ± 2.6 for Leu$^-$ segregants; none were lower than 6.9]. That all of the tetrads in the *mof9* × rDNA::LEU2 cross segregated 4 high:0 low demonstrates that *mof9* is genetically linked to rDNA at the *RDN1* locus. By inference, the Leu$^-$ segregants were all *mof9*. Three complete tetrads of cross JD746 were also analyzed for β-galactosidase activities and no significant differences were observed (115 ± 17 for rDNA::LEU2, 115 ± 16 for *mof9*). The efficiencies of −1 ribosomal frameshifting for rDNA::LEU2 and *mof9* cells in this cross were thus 8.9 and 8.3%, respectively.

When transformed with pFS22 or pJD64, CEN plasmids carrying 5 S rDNA (Figure 2), the frameshifting efficiencies of the rDNA::LEU2 strains returned to 1.8 and 2.0%, respectively. Providing the 5 S rDNA on a multi-copy vector (pJD106) was equally able to complement both the *mof9* and rDNA::LEU2 mutations (Figure 2 and Table 4), neither of these plasmids had any effect upon frameshifting efficiencies of wild-type cells. There were no significant effects of temperature upon −1 ribosomal frameshifting (data not shown).

The Mof9$^-$ phenotype can be reproduced by expression of mutant 5 S rRNAs: *mof9*, *rDNA::URA3* and wild-type cells containing either p0 or p−1 were transformed with the high copy plasmids, pYF404 (vector only), pYF404Y5 (5 S rDNA clone), pYF404Y5G98 (5 S rDNA C98 → G mutation), and pYF404Y5A99 (5 S rDNA G99 → A mutation), and the ratios of their β-galactosidase activities were determined (Table 5A). In wild-type cells, overexpression of 5 S rRNA had no effect upon frameshifting efficiencies, but overexpression of the G98 and A99 mutants increased −1 ribosomal frameshifting efficiencies 1.4- and 2.2-fold, respectively. Overexpression of wild-type 5 S rRNA in *mof9* and *rDNA::URA3* cells corrected the *mof9*$^-$ phenotype, but overexpression of

TABLE 3

Frameshifting in *rDNA::URA3* and *rDNA::LEU2* integrative recombinants

Strain	Genotype	β-galactosidase activity: 0 frame[a]	β-galactosidase activity: −1 frame[b]	% −1 frameshift
A. *trp1* spore clones containing p0 and p − 1 from crosses 4377 and 4378				
4377-8C	*rDNA::LEU2*	132 ± 34	14.5 ± 0.8	11
4377-1B	*rDNA::LEU2*	73 ± 2.5	4.4 ± 0.0	6
4378-5D	*rDNA::URA3*	202 ± 20	21 ± 4.4	10.4
4378-9B	*rDNA::URA3*	62 ± 1.3	4.6 ± 2.2	7.4
4377-8B	*rDNA*$^+$	48 ± 3.1	0.9 ± 0.2	1.9
4377-11B	*rDNA*$^+$	63 ± 5.8	1.1 ± 1.7	1.7
4378-5B	*rDNA*$^+$	75 ± 1.7	1.3 ± 0.0	1.8

Strains	Genotype	β-galactosidase activity: 0 frame[a]	β-galactosidase activity: −1 frame[b]	% frameshift
B. Complementation Tests				
JD18-3C × 2907	*mof9/MOF*$^+$	177 ± 6.7	4.0 ± 0.0	2.4%
4377-8C × 2907	*rDNA::LEU2/MOF*$^+$	180 ± 7.2	3.6 ± 0.5	2.0%
EMS56 × 4378-5D	*mof9/rDNA::URA3*	161 ± 9.0	13 ± 3.2	8.1%
JD18-3C × 4378-6B	*mof9/rDNA::URA3*	68 ± 3.7	6.3 ± 1.4	9.3%

745−[c]	Activity[b]	Leu	745−[c]	Activity[b]	Leu	746−[d]	Activity[b]	Leu
C. Cosegregation of *mof9* and *rDNA::LEU2*								
1A	11.5	−	4A	8.4	−	1A	91	−
1B	11.5	+	4B	8.2	+	1B	110	−
1C	10.5	+	4C	6.9	−	1C	134	+
1D	12.5	−	4D	7.5	+	1D	164	+
2A	8.5	−	5A	13.3	+	2A	153	−
2B	12.3	+	5B	15.6	−	2B	186	+
2C	7.4	+	5C	12.2	+	2C	151	+
2D	7.8	−	5D	8.7	−	2D	176	−
3A	9.4	−	6A	10.0	−	3A	86	−
3B	14.0	−	6B	8.7	−	3B	72	+
3C	10.8	−	6C	11.4	+	3C	122	+
3D	10.9	+	6D	7.1	+	3D	72	−

Crosses	Segregants	0 frame[a]	−1 frame[b]	% frameshift
JD745[c] & JD746[d]	*rDNA::LEU2*	115 ± 18	10.2 ± 1.9	8.9
	mof9	115 ± 16	9.6 ± 2.4	8.3

[a] β-galactosidase measured in cells carrying p0.

[b] β-galactosidase measured in cells carrying p−1.

[c] Cross JD745 was EMS56 (*mof9-1*) × 4377-8C (*rDNA::LEU2*) with all segregants carrying p−1. Twenty-four segregants were analyzed, of which 12 carried *rDNA::LEU2* and 12 were inferred to be *mof9*.

[d] Cross JD746 was EMS56 (*mof9-1*) × 4377-8C (*rDNA::LEU2*) with all segregants carrying p0. Twelve segregants were analyzed, of which six carried *rDNA::LEU2* and six were inferred to be *mof9*.

the G98 or A99 mutants further increased the efficiencies of −1 ribosomal frameshifting 1.4- to 1.9-fold in these cells. VAN RYK *et al.* (1990) showed that on minimal medium, in wild-type cells carrying these plasmids, 50–80% of the 5 S rRNA is of the mutant form.

We constructed pJD116Y5, pJD116Y5C98G and pJD116Y5G99A carrying the same wild-type and mutant alleles as in the pYF plasmids obtained from R. NAZAR. The wild-type 5 S rRNA gene on pJD116Y5 had no effect on frameshifting in a wild-type host but corrected the elevated frameshifting in *mof9* cells (Table 5B). Expression of the C98G mutant increased frameshifting 2.1- to 2.5-fold in wild-type cells, while the G99A mutant had a 2.9- to 3.4-fold effect in the same cells (Table 5B). We confirmed the results of VAN RYK *et al.* (1990) that a substantial fraction of total 5 S rRNA was the mutant form (see below).

The *Mof9*$^-$ phenotype is best observed in the context of high-copy number reporter genes: An *rDNA::URA3* strain was mated to wild-type strains containing the integrated pJD85::leu2-1 (−1 ribosomal frameshift indicator) or pJD86::leu2-1 (0-frame control) (DINMAN and

TABLE 4

5S rDNA on single or multiple copy vector complements the *mof9* and *rDNA::LEU2* mutants

Genotype	−1 ribosomal frameshifting efficiency (%)[d]		
	None	+5S-CEN[e]	+5S-high copy[f]
mof9[a]	5.2	1.9	1.9
rDNA::LEU2[b]	6.0	2.0	1.8
Wild-type[c]	1.8	2.1	2.0

[a] EMS56, [b] 4377-8C and [c] 4377-8B strains were used in this experiment.

[d] Each strain carried p−1, with lacZ in the −1 frame relative to the start codon, or p0.

[e] Plasmid pJD64.

[f] Plasmid pJD106.

TABLE 5

The *Mof9*⁻ phenotype is reproduced by overexpression of mutant 5S rRNAs

Plasmid	*MOF+*[a]	*mof9*[b]	*rDNA::URA3*[c]
A. % −1 ribosomal frameshifting			
None	1.6	4.3	6.8
pYF404	1.7	4.7	7.0
pYF404Y5	1.9	2.0	2.0
pYF404Y5G98	2.4	6.4	9.3
pYF404Y5A99	3.7	6.6	12.7
Plasmid	*MOF+*[a]	*MOF+*[d]	*mof9*[e]
B. % −1 ribosomal frameshifting			
pJD116Y5	1.9	2.0	2.0
pJD116Y5C98G	3.9	5.0	6.7
pJD116Y5G99A	6.5	5.8	5.6

All cells were transformed with p−1 or p0 and then transformed with the pYF404 plasmid series.

[a] Strain 2907, [b] strain JD18-7A, [c] strain 4378-5D, [d] strain 4378-5B, [e] strain EMS56.

WICKNER 1994) and the *LEU*⁺ progeny (carrying the integrated pJD85 and pJD86 constructs) were assayed for β-gal activities. The β-galactosidase activities of cells containing pJD86 (0-frame controls) were 7.6 ± 2.2 (URA^+) and 6.5 ± 1.0 (Ura^-) and the β-galactosidase activities of cells containing pJD85 were 0.23 ± 0.04 (URA^+) and 0.14 ± 0.04 (Ura^-). The −1 ribosomal frameshifting efficiencies were 3.0 and 2.2% in rDNA::URA3 spore clones and wild-type spore clones, respectively, a difference of only 1.4-fold, *i.e.*, only approximately half the increase seen when −1 ribosomal frameshifting efficiencies were measured from high copy, episomal vectors.

Molecular analyses: To determine if differences in plasmid copy number were responsible for the effects observed, DNA was extracted from rDNA disruptants, *mof9* and wild-type cells containing p−1 and p0. Serial dilutions of equal quantities of total DNA were loaded onto a filter in a slot blot format, hybridized with [αP^{32}]-dCTP labeled pBR322 and filters were exposed to X-ray film. Autoradiographs of these filters showed no significant differences in signal intensity between mutant and wild-type for either p−1 or p0 (data not shown). Likewise, Southern analysis of 5 S rDNA gene arrangement showed no significant differences between mutant and wild-type cells although the differences between form I and form II rDNAs could be discerned (data not shown). The observed phenotype of the *mof9* and rDNA integrative recombinants thus cannot be explained by differences in reporter plasmid copy numbers or by some gross gene rearrangement in the majority of the rDNA repeats.

Total RNA was extracted from an equal number of *mof9, rDNA::LEU2* and wild-type control cells, separated through a 2% agarose gel and transferred to a nylon membrane. This blot was probed with a (−) strand probe (T7 RNA polymerase transcript of *Cla*I-restricted pJD64) to detect differences in 5 S RNA hybridizing signals. No differences were found (data not shown). To look for antisense transcripts that may interfere with 5 S rRNA function in these mutants, the blot was stripped of probe and reprobed with a (+) strand probe (T3 RNA polymerase transcript of *Bam*HI-restricted pJD64). No hybridizing bands were detected (data not shown). To detect small differences in 5 S rRNAs, nucleic acids were labeled *in vivo* with carrier free [^{32}P]-orthophosphate, the RNA was extracted and separated an 8% polyacrylamide denaturing gel. No mobility differences in 5 S RNAs were detected (data not shown). The 5 S and 35 S RNA species are expressed independently of one another, but their expression is in balance (NEIGEBORN and WARNER 1990). Optical scanning densitometry was employed to determine the ratios of 5 S and 5.8 S species in mutant and wild-type cells in an attempt to see if the regulation of these species might be unbalanced in the mutants. After subtracting background and normalizing for specific activities [yeast 5 S rRNA is 121 nucleotides in length (MAXIM *et al.* 1977, OLSON *et al.* 1977, VALENZUELA *et al.* 1977) and the 5.8 S rRNA is 158 nucleotides long (RUBIN 1973)], we found no significant differences in the ratio of levels of 5 S to 5.8 S rRNAs comparing wild-type and mutant cells (data not shown). Samples were also separated through an 8% polyacrylamide native gel in an attempt to detect mobility shifts in 5 S rRNAs due to conformational differences (VAN RYK *et al.* 1990). No such shifts were detected (data not shown). Polysome profiles of *mof9* strains were normal (data not shown).

Purified 5 S rRNA isolated from cells containing pJD116Y5, pJD116Y5C98G and pJD116Y5G99A was 5′ end-labeled and separated through a 12% acrylamide gel under native conditions as described by VAN RYK *et al.* (VAN RYK and NAZAR 1992) and visualized by autoradiography (Figure 4). The mobilities of the C98G and G99A mutant 5 S rRNAs matched those seen by VAN

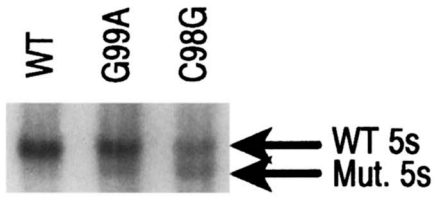

FIGURE 4.—Plasmid-expressed mutant 5 S rRNAs comprise 20–50% of total cellular 5 S rRNA. 5 S rRNA was purified from wild-type strain 2907 expressing wild-type 5 S rRNA from pJD116Y5, mutant G99A from pJD116Y5G99A or mutant C98G from pJD116Y5C98G. Isolated 5 S rRNAs were 5′-labeled with [γ-^{32}P]ATP and polynucleotide kinase and analyzed by polyacrylamide gel electrophoresis as described by Van Ryk *et al.* (1992) (see MATERIALS AND METHODS). The autoradiogram is shown.

RYK *et al.* and the results showed that 20–50% of total cellular 5 S rRNA was of mutant form.

The lacZ reporter mRNA produced from p−1 can be seen as a nonsense mRNA, *i.e.*, it presents the cellular translational machinery with a short 0 frame open reading frame that is quickly terminated. Nonsense mRNAs are typically degraded by the nonsense-mediated mRNA decay pathway, and mutants in this pathway, having increased half-lives of nonsense mRNAs, have been characterized (PELTZ *et al.* 1994). The identification of *mof9* is based upon increased β-galactosidase activity in these cells, presumably due to an increase in the efficiency of −1 ribosomal frameshifting. If the *mof9* mutation were the result of a mutation in a gene involved in the nonsense-mediated mRNA decay pathway, the longer half-life of the reporter mRNA might also result in the accumulation of a greater amount of β-galactosidase. Thus, the same result, *i.e.*, an increase in β-galactosidase activity, could be observed in either case.

To test this, the relative amounts of steady state β-galactosidase mRNAs were determined by RNA-RNA hybridization in wild-type and *mof9* cells containing p0 and p−1. No differences in the relative signal intensities were observed between wild-type and *mof9* cells with either p0 or p−1, indicating that the Mof9 phenotype is not due to a mutation in the nonsense-mediated mRNA decay pathway (data not shown).

Specificity of *mof9*: Both *mof9* cells and *rDNA::LEU2* cells showed increased −1 frameshifting with several different slippery sites (Table 6). A similar effect was observed on each of the slippery sites tested. A +1 shift promoted by the Ty1 retrotransposon site that determines +1 frameshifting (BELCOURT and FARABAUGH 1990) was also more shifty in both *mof9* cells and *rDNA::LEU2* cells than in wild-type cells (Table 6). However, no effect was seen with a frameshift signal having a disrupted pseudoknot.

Termination readthrough was measured using pJD115a and pJD115o, which have amber and ochre mutations, respectively, at the A site of the slippery site and the *lacZ* gene in the 0 frame. There was no elevation of readthrough with either vector in *mof9, rDNA::URA3* or *rDNA::LEU2* host strains, indicating that the observed mutant effects were specific to maintenance of frame and not a general inaccuracy of translation.

DISCUSSION

We have shown that mutations in the rDNA locus increase the efficiency of −1 and +1 ribosomal frameshifting in yeast. Three independently generated mutations, *i.e.*, the EMS-generated *mof9* mutation, as well as the *rDNA::LEU2* and *rDNA::URA3* insertion disruptions, result in the Mof$^-$ phenotype. Each is apparently semirecessive, and they do not complement one another or segregate in meiosis, indicating that the lesions are at the same genetic locus. Further, each of these mutations are complemented by plasmid-borne 5 S rDNA clones, either on single or high copy vectors. In addition, expression of either of two mutant 5 S RNAs characterized by TANG and NAZAR (1992) produced the Mof$^-$ phenotype in a wild-type strain, again showing that 5 S rRNA is involved in maintenance of reading frame.

How could a mutation of one or a few of the many 5 S rDNA genes result in a phenotype? There are 100–200 rDNA genes per haploid yeast genome all of which are located in a single tandem array on chromosome *XII*, the *RDN1* locus (PETES 1979a,b). Nazar's work (VAN RYK *et al.* 1990) shows that altering the structure around the 5 S rRNA gene dramatically increases its expression. This may explain why alteration of one or a few copies in the rDNA locus affecting 5 S rRNA may have a disproportionate effect. We found that the normal 5 S rRNA gene on the CEN vector YCp50 restored the efficiency of frameshifting in *mof9* cells to nearly normal levels. In confirmation of the work of Nazars group (VAN RYK *et al.* 1990), we find that plasmid-expressed 5 S rRNA can amount to up to half of total 5 S rRNA in the cell. We do not yet know the precise defect in the original *mof9* mutant, but the fact that the mutation was located in the rDNA locus as shown by genetic crosses, was complemented by the 5 S rRNA gene, and that a similar defect was induced in a wild-type host by the overexpression of a mutant 5 S rRNA gene all show that 5 S rRNA has a role in maintenance of reading frame.

Several factors point toward the uniqueness of the *RDN1* locus. Although it consists of a large array of tandemly repeated rDNA genes, the frequency of meiotic recombination between them is suppressed by a factor of ≥15 (ZAMB and PETES 1982). The 5 S rDNA genes are located within the nontranscribed (by RNA polymerase I) spacer region and are situated between the rDNA enhancer region and the 35 S rRNA transcriptional start site (ELION and WARNER 1986). The 5 S rDNA gene is transcribed by RNA polymerase III from the opposite DNA strand (PHILIPPSEN *et al.* 1978). Expression of 5 S RNA is independent of the region responsible for enhancement of 35 S RNA transcription,

TABLE 6

Effect of *mof9* and *rDNA::LEU2* mutations on different slippery sites

Plasmid	Slippery site	Host strain		
		WT[a]	*mof9*[b]	*rDNA::LEU2*[c]
		% frame shifting		
pF8	GGGTTTA	2.2	7.0 (3.2×)	3.2 (1.6×)
pJD32	TTTTTTA	8.1	18.6 (2.3×)	7.9 (1×)
pJD11	AGGTTTA	0.1	0.3 (3×)	0.4 (4×)
pJD28	TTTGGGC	0.1	0.3 (3×)	0.3 (3×)
pJD30	GGGTTTG	0.2	0.7 (3.5×)	1.1 (5.5×)
pJD18	5′cPsi	0.1	0.1 (1×)	0.1 (1×)
pJD104	Ty1 +1 Site	1.8	4.9 (2.7×)	4.5 (2.5×)

Plasmid	Slippery site	Host strain				
		WT[d]	*mof9*[e]	WT[f]	*rDNA::LEU2*[c]	*rDNA::URA3*[g]
		% frame shifting				
pJD115a	GGGTTAGA-lacZ in 0 frame	0.05	0.06	0.06	0.06	0.04
pJD115o	GGGTTAAA-lacZ in 0 frame	0.04	0.06	0.09	0.08	0.03

[a] Strain 4377-8B; [b] strain JD18-3C; [c] strain 4377-8C; [d] strain 2907; [e] strain EMS56; [f] strain 4378-5B; [g] strain 4378-9B. Plasmids pJD32, pJD11, pJD28, pJD30, pJD115a and pJD115o have the same L-A-derived sequences as pF8 except for the indicated alteration of the slippery site (DINMAN *et al.* 1991). pJD18 has a disruption of the pseudoknot structure but a normal slippery site (DINMAN *et al.* 1991). pJD104 (BALASUNDARAM *et al.* 1994) has the Ty1 +1 slippery site (BELCOURT and FARABAUGH 1990) inserted in place of the normal L-A −1 slippery site along with one extra codon to separate it from the pseudoknot. pJD115a and pJD115o have amber (TAG) and ochre (TAA) codons in the 0-frame in the slippery sites and an extra nucleotide to put the lacZ reporter gene in the 0-frame with regard to the translational start site. Thus, pJD115a and pJD115o measure readthrough of stop codons and not ribosomal frameshifting. Percentage values are β-galactosidase activity relative to the 0 frame plasmid, pTI25. "×" values in parentheses show the frameshifting of mutant/wild-type.

and it has been proposed that the 5 S rDNA gene is looped out by specific interactions between the enhancer and the 35 S rRNA promoter (NEIGEBORN and WARNER 1990). It has been shown that plasmid-borne mutant 5 S RNAs are preferentially assembled into an *in vivo* ribosome population (VAN RYK *et al.* 1990), and here we have shown that these mutant 5 S rRNAs can manifest themselves by a Mof⁻ phenotype. The preferential incorporation of plasmid-borne 5 S rRNAs may also explain why the frameshifting phenotypes of the *mof9*, *rDNA::LEU2* and *rDNA::URA3* chromosomal mutations are corrected by pJD64 and pJD106. The derepressed transcription of plasmid-borne 5 S rDNA genes suggests that the nucleolar environment of the rDNA locus differs critically from the presumably nonnucleolar environment of a plasmid. Perhaps the *rDNA::LEU2* and *rDNA::URA3* insertion mutations locally disrupt this environment leading to production of altered 5 S RNA, perhaps in amounts out of proportion to the number of 5 S genes affected. Alternatively, the 5 S rRNA encoded by these strains may be a natural variant that inherently supports a higher frameshift efficiency even without the insertion mutations.

How might 5 S rRNA affect maintenance of reading frame? 5 S rRNA associates with the large ribosomal protein L1 (also known as YL3) (NAZAR *et al.* 1979, TANG and NAZAR 1991). 5 S rRNAs from HeLa and Xenopus cells compete less effectively than that from *Saccharomyces cerevisiae* for their ability to complex with *S. cerevisiae* L1 and TFIIIA even though they differ at only 13 of 120 positions (BROW and GEIDUSCHEK 1987), implying that changes in 5 S rRNA sequence can affect its ability to complex with L1. Mutations in the nontranscribed region of the rDNA repeat 3′ of the 5 S coding sequence have been shown to produce altered transcripts of yeast 5 S rRNA that bind less stably to L1 (BROW 1987). Transcription of 5 S rRNA can also be influenced by the nontranscribed sequence 5′ of the 5 S coding region (MORTON and SPRAGUE 1984). These observations may explain how the *rDNA::URA3* and *rDNA::LEU2* integrative recombinant constructs used in the present study (see Figure 2) (PETES *et al.* 1978, PETES 1980) were able to show the Mof⁻ phenotype.

The L1-5 S rRNA ribonuclear particle (RNP) is stable in the absence of 60 S ribosomal subunit assembly but 5 S rRNA is unstable upon depletion of L1 (DESHMUKH *et al.* 1993). Yeast cells contain a pool of L1-5 S rRNA not associated with ribosomes, perhaps located in the nucleus (DESHMUKH *et al.* 1993). In Xenopus, 5 S rRNA is assembled into 60 S ribosomal subunits in the nucleus, and extensive mutational analysis has shown that the structural requirements for assembly of the L1-5 S

rRNA RNP, and for its incorporation into 60 S ribosomal subunits, are complex and nonidentical (ALLISON *et al.* 1993). The Loop E region of 5 S rRNA (NAZAR 1991, NAZAR *et al.* 1991), which contains the C98 → G and G99 → A mutations used in this study, is implicated in ribosome incorporation and protein recognition of noncanonical base-pairing and may be important for incorporation into the 60 S ribosomal subunit and RNA-RNA hybridization (ALLISON *et al.* 1993). VAN RYK and NAZAR (1992) note that the 5 S rRNA has some flexibility as defined by susceptibility and resistance to digestion by ribonucleases and alkylating agents. The G99 → A mutation, which allows formation of an A:U base pair, is less flexible than wild-type, and the C98 → G mutation, which breaks a G:C base pair, is more flexible. Changes in 5 S rRNA flexibility could affect its ability to interact properly with large subunit proteins, *e.g.*, L1, affecting overall ribosomal fidelity. Thus, it is possible that the observed impairment in maintenance of frame in some cases is due to effects of the 5 S rRNA mutation on the assembly and structure of the ribosome rather than to the direct action of the 5 S rRNA itself.

The competitive-displacement model of SARGE and MAXWELL (1991) implicates 5 S rRNA in translational initiation via its ability to base pair to 18 S rRNA on the 40 S ribosomal subunit. In this model, the KOZAK consensus region of an mRNA binds to a region in the 18 S rRNA (region 1), preventing base pairing of initiator $tRNA^{Met}$ in the 48 S preinitiation complex. The 60 S ribosomal subunit docks to this complex and a region in the 5′ terminal sequence (nucleotides 6–27 in mouse 5 S rRNA) is able to base pair to the 18 S rRNA region 1, displacing the 18 S rRNA/mRNA hybrid, thus allowing the initiator $tRNA^{Met}$ to base pair to the AUG start site and initiate translation. VAN RYK and NAZAR (1992) found that the C98 → G mutation affected the ability of T_1 ribonuclease to cleave at several sites, including after G25, which is in the region of 5 S rRNA hypothesized to be involved in base pairing with 18 S rRNA. Comparison of the nuclease sensitive sites in the helix IV mutants of the yeast C98 → G and G99 → A 5 S rRNA (VAN RYK and NAZAR 1992) with the mapping of sites involved in TFIIIA binding and ribosome incorporation in Xenopus 5 S rRNA (ALLISON *et al.* 1993) shows that these mutations could have potential effects on both of these functions. Given the interaction of 5 S rRNA and L1, it is reasonable to anticipate that some mutant alleles of L1 may yield a Mof^- phenotype. Such analyses may provide a more detailed understanding of the roles of specific ribosomal proteins and rRNAs in the fidelity of the translational process.

The authors thank ROSS NAZAR for providing the pYF404 series of plasmids. We thank TOM PETES for providing *rDNA::URA3* and *rDNA::LEU2* yeast strains and greatly appreciate his hard work in deducing the structures of the integrative recombinant plasmids with which these strains were originally constructed.

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Communicating editor: M. JOHNSTON