Translational Maintenance of Frame: Mutants of *Saccharomyces cerevisiae* With Altered −1 Ribosomal Frameshifting Efficiences

Jonathan D. Dinman and Reed B. Wickner

Laboratory of Biochemical Pharmacology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Manuscript received May 5, 1993
Accepted for publication September 16, 1993

ABSTRACT

A special site on the (+) strand of the L-A dsRNA virus induces about 2% of ribosomes translating the *gag* open reading frame to execute a −1 frameshift and thus produce the viral *gag-pol* fusion protein. Using constructs in which a −1 ribosomal frameshift at this site was necessary for expression of *lacZ* we isolated chromosomal mutants in which the efficiency of frameshifting was increased. These mutants comprise eight genes, named *mof* (*m*aintenance *of* frame). The *mof1-1*, *mof2-1*, *mof4-1*, *mof5-1* and *mof6-1* strains cannot maintain M_1 dsRNA at 30°, but, paradoxically, do not lose L-A. The *mof2-1*, *mof5-1* and *mof6-1* strains are temperature sensitive for growth at 37°, and all three show striking cell cycle phenotypes. The *mof2-1* strains arrest with mother and daughter cells almost equal in size, *mof5-1* arrests with multiple buds and *mof6-1* arrests as single large unbudded cells. *mof2-1* and *mof5-1* strains are also Pet⁻. The *mof* mutations show differential effects on various frameshifting signals.

ALTHOUGH maintenance of the correct reading frame is fundamental to the integrity of the translation process, a number of cases of "purposeful" frameshifting have been identified, including retroviruses, coronaviruses, the L-A dsRNA virus of yeast, (+) ssRNA viruses of plants, bacteriophage T7, bacterial transposons and a few bacterial cellular genes (for reviews see HATFIELD *et al.* 1992; JACKS 1990; ATKINS *et al.* 1991; CHANDLER and FAYET, 1993). These frameshifting events produce fusion proteins, whose N- and C-terminal portions are encoded by two distinct (overlapping) open reading frames. The same result could be achieved by splicing or editing of the RNA. However, for the retroviruses, dsRNA viruses and (+) ssRNA viruses, which use their (+) strands as mRNA, as the species packaged to make new virions and as a template for replication, either splicing or editing would produce mutant virus unless the splicing events removed an RNA site necessary for packaging or replication of the RNA. Perhaps for this reason (ICHO and WICKNER 1989), (+) ssRNA and dsRNA viruses are not known to use splicing or editing of their RNA and retroviruses remove the packaging site (called ψ) when they splice their RNA so that the spliced RNA is not propagated. All three classes of virus use ribosomal frameshifting and/or readthrough of termination codons to make fusion proteins. Neither of these mechanisms alters the template and so neither produces mutant virus.

The −1 frameshift events require a special sequence at the site of the frameshift (the slippery site) of the form X XXY YYZ, where the unshifted frame is indicated (JACKS *et al.* 1988). Simultaneous slippage of the A-site and P-site tRNAs by 1 base toward the 5′ end of the mRNA leaves their non-wobble bases correctly paired. A second, promoting element, is also necessary for efficient −1 shifting (JACKS *et al.* 1988). This is generally an RNA pseudoknot located just 3′ to the slippery site (BRIERLEY, JENNER and INGLIS 1992; TEN DAM, PLEIJ and DRAPER 1992), whose function is apparently to slow the ribosome movement (TU, TZENG and BRUENN 1992) and favor backward movement. The efficiency of −1 ribosomal frameshifting is determined by (i) how often the tRNA (particularly the A-site tRNA) unpairs from the mRNA (DINMAN, ICHO and WICKNER 1991; BRIERLEY, JENNER and INGLIS 1992), (ii) how well the A- and P-site tRNAs can re-pair with the mRNA in the −1 frame (JACKS *et al.* 1988), and (iii) by the strength and position of the 3′ pseudoknot (BRIERLEY, DINGARD and INGLIS 1989; BRIERLEY *et al.* 1991; DINMAN and WICKNER 1992).

The 4.6-kb dsRNA L-A virus of *Saccharomyces cerevisiae* has two open reading frames, *gag*, which encodes its major coat protein, and *pol*, encoding a multifunctional protein domain that includes the RNA-dependent RNA polymerase and a domain necessary for packaging of viral RNA (HOPPER *et al.* 1977; ICHO and WICKNER 1989; RIBAS and WICKNER 1992; FUJIMURA *et al.* 1992). *pol* is synthesized only as a *gag-pol* fusion protein formed by a −1 ribosomal frameshift event (FUJIMURA and WICKNER 1988; ICHO and WICKNER 1989; DINMAN, ICHO and WICKNER 1991). M_1, a satellite dsRNA of L-A, is encapsidated and replicated

by the L-A-encoded proteins, and encodes a secreted toxin, the killer toxin. We have shown that the efficiency of the −1 ribosomal frameshift event is critical to support the propagation of the M_1 satellite dsRNA from an L-A cDNA clone (DINMAN and WICKNER 1992). Whether frameshifting efficiency was altered by mutations in the slippery site sequence, by substituting a +1 frameshifting signal of the type utilized by the yeast retrovirus Ty*1* (BELCOURT and FARABAUGH 1990) or by a chromosomal mutation (*hsh1*), increasing or decreasing frameshift efficiency more than 2-fold away from the normal 1.9% resulted in loss of the M_1 satellite dsRNA. Similarly, decreased efficiency of Ty*1*'s +1 ribosomal frameshifting likewise decreases its transposition frequency (XU and BOEKE 1990). These findings led us to suggest that ribosomal frameshift efficiency might be a useful target for antiviral drugs.

Synthesis of the viral replicase in the form of a fusion protein insures its inclusion in the viral particles and, at least for the case of the L-A dsRNA virus, the fusion protein has a role in the packaging mechanism. The N-terminal ¼ of the *pol* domain of the *gag-pol* fusion protein is necessary for packaging and includes an RNA binding site (FUJIMURA *et al.* 1992; J. C. RIBAS, T. FUJIMURA and R. WICKNER, unpublished results). This suggests that this packaging region of *pol* holds onto the viral (+) strands while the *gag* domain associates with free *gag* protein to produce packaging of viral RNA in new particles in a very natural way (FUJIMURA and WICKNER 1988). This could explain why frameshift efficiency is so critical (DINMAN and WICKNER 1992). Too many *gag-pol* fusion molecules relative to *gag* molecules might result in the initiation of assembly of many new particles with insufficient *gag* protein to finish any of them. A relative excess of *gag* might result in frequent completion and closure of particles before the *pol* domain of the fusion protein has had a chance to find a viral (+) strand. Alternatively, since there are about two fusion proteins per particle, dimer formation by these might become rate-limiting at low fusion protein levels (DINMAN and WICKNER 1992).

The importance of maintenance of reading frame in both normal translation and in viral ribosomal frameshifting led us to initiate a study of chromosomal genes affecting this process. We report the identification and analysis of eight genes, mutations in which increase the efficiency of ribosomal frameshifting by the L-A frameshifting signal.

MATERIALS AND METHODS

Strains and media: The strains of *S. cerevisiae* used are listed in Table 1. YPAD, YPG, SD, complete synthetic medium (H-trp, -leu) and 4.7MB plates for testing the killer phenotype were as previously reported (WICKNER and LEIBOWITZ 1976). *Escherichia coli* strain JM109 was used for production of plasmid DNA.

Genetic methods: Transformation of yeast was by the lithium acetate method (ITO *et al.* 1983) and of *E. coli* was by the calcium chloride method (MANIATIS, FRITSCH and SAMBROOK 1982). Genetic crosses, sporulation, and tetrad analyses were performed as described previously (SHERMAN 1991; WICKNER 1991). The killer test was performed by replica plating colonies to be tested to 4.7MB plates with a newly seeded lawn of toxin-sensitive strain 5X47 (0.5 ml of a 1 OD_{550}/ml suspension per plate). After 2 days at 20°, killer activity was observed as a clear zone around the killer colonies. Mitochondrial DNA was eliminated by streaking for single colonies on H-leu plates containing 1 μg of ethidium bromide per ml. Cytoduction (cytoplasmic mixing without nuclear fusion) was carried out as described (RIDLEY, SOMMER and WICKNER 1984). The donor *kar1* strain (1074, 2631 or RE406), which is defective in nuclear fusion (karyogamy) and which harbors the L-A and M_1 or X dsRNA viruses, was mixed with the recipient strain which lacked M_1, X and mitochondrial DNA. The mixtures were incubated on YPAD plates for 6 hours at 30° or for 16 hr at 20° and plated on medium lacking leucine to select against the donor strain. Clones were checked for growth on minimal medium (to screen out diploids) and on glycerol medium (to check for receipt of the donor cytoplasm, including the mitochondrial genome) and for the killer phenotype (as evidence of propagation of M_1). Several [ψ^+] strains were cured of [ψ] by streaking on YPAD medium containing 5mM guanidine (TUITE, MUNDY and COX 1981). Loss of [ψ] was confirmed by loss of suppression of the ade2-1 mutation. Temperature-resistant revertants of *mof2-1*, *mof5-1* and *mof6-1* cells were identified by plating approximately 8×10^7 cfu on H-leu medium and selecting for growth at 37°. Resulting colonies were streaked for single colonies at 37° and assayed for β-galactosidase activity (see below).

Plasmids: Plasmids pTI25, pF8, pJD11, 12, 13, 18, 23, 24, 26, 28, 29, 30, 31, 32, 58 and 70 have been described (DINMAN, ICHO and WICKNER 1991; DINMAN and WICKNER 1992). Briefly, pTI25 is the 0-frame control plasmid, while pF8 is the −1 ribosomal frameshift indicator plasmid. pJD29 is identical to pF8 except that it has GGGTTTT as its slippery site in place of the normal GGGTTTA. The rest of plasmids pJD11 through pJD70 contain alterations in the frameshift site or downstream pseudoknot (see Table 7). pCC10 and pCC10* (ENG, PANDIT and STERNGLANZ 1989) were generous gifts from R. STERNGLANZ. pJD84 was constructed as follows: plasmid pRS305 (SIKORSKI and HIETER 1989) was partially cleaved with *Cla*I, and linearized 5.5-kb plasmid was gel purified. Overhanging 5′ ends were filled with Klenow fragment, the blunt ends were religated and the mixture used to transform *E. coli* JM109. Restriction analysis was used to identify pJD84 by the loss of the *Cla*I site in the polylinker instead of in the *LEU2* gene. The YIp plasmids pJD85 and pJD86 were made as follows: pF8 and pTI25 were cleaved with *Hin*dIII and the 4.9-kb (pF8) and 4.7-kb (pTI25) fragments were gel purified. These were ligated into *Hin*dIII cleaved pJD84 to create pJD85 (the −1 frameshift YIp tester plasmid) and pJD86 (the 0-frame control YIp tester plasmid). The unique *Cla*I site in the *LEU2* gene of pJD85 and pJD86 was then used to direct integration of the constructs to the chromosomal *leu2-1* locus (see below). The constructs are denoted *leu2-1*::pJD85 and *leu2-1*::pJD86, but both are Leu$^+$ because of the *LEU2* gene of the integrating plasmids.

Measurement of −1 frameshifting and β-galactosidase assays: Frameshifting was measured by comparing the β-galactosidase activities from two constructs. In one, the

TABLE 1

Strains and crosses of *S cerevisiae*

Strain	Genotype	Source
5X47	*MATa/MATα his1/+ trp1/+ ura3/+* K^-R^-	
10	*MATa his4-644 leu2-1*	
JD348	*MATa his4-644 leu2-1*::pJD85[a]	Transformation of strain 10
JD349	*MATa his4-644 leu2-1*::pJD86[a]	
JD272	*MATα leu2⁻ lys11 trp1Δ ura3* K_1^-	
JD63-17D	*MATα his4 ura3 leu2-1*::pJD85	Segregant of JD348 × JD272
JD64-4D	*MATα trp1 lys11 leu2-1*::pJD86	Segregant of JD349 × JD272
JD52	*MATa his3-δ200 leu2⁻ trp1-Δ901 ura3-52 ade2-10* K^-	K. MATSUMOTO
JD404	*MATa leu2-1*::pJD85 *his4-644 ura3 ade2 MOF⁺*	=JD75-1D
JD595	*MATa leu2-1*::pJD85 *lys2-187 met8-1 aro7-1 his4-166 ura3 SUP42* ×*MATα leu2-1*::pJD85 *lys2-187 met8-1 aro7-1 ura3 trp1*	
JD596	*MATα leu2-1*::pJD85 *lys2-187 met8-1 aro7-1 his4-166 trp1 ura3 SUP46* × *MATa leu2-1*::pJD85 *met8-1 aro7-1 lys2-187 ura3*	
	***mof* mutants**	
EMS46	*MATa his3-d200 leu2⁻ trp1-d901 ura3-52 ade2-10 mof1* K^-	
JD67-1C	*MATα leu2-1*::pJD85 *trp1-d901 ade2 his3,4 ura3 mof1*	Segregant of EMS46 × JD63-17D
EMS 41	*MATa his3-d200 leu2⁻ trp1-d901 ura3-52 ade2-10 mof3-1* K^-	
JD65-10B	*MATα leu2-1*::pJD85 *trp1d901 ura3 MOF+*	Segregant of EMS41 × JD63-17D
Cross JD75	JD348 × JD67-1C	
Cross JD68	EMS46 × JD64-4D	
Cross JD66	EMS 41 × JD64-4D	
Cross JD477	EMS133 *MATα leu2-1*::pJD85 *trp1d901 ura3 mof2* × JD404	
Cross JD474	EMS293 *MATα leu2-1*::pJD85 *trp1d901 ura3 mof4* × JD404	
Cross JD468	EMS136 *MATα leu2-1*::pJD85 *trp1d901 ura3 mof5* × JD404	
Cross JD469	EMS162 *MATα leu2-1*::pJD85 *trp1d901 ura3 mof6* × JD404	
Cross JD471	EMS235 *MATα leu2-1*::pJD85 *trp1d901 ura3 mof7* × JD404	
Cross JD472	EMS255 *MATα leu2-1*::pJD85 *trp1d901 ura3 mof8* × JD404	

[a] *leu2-1*::pJD85 and *leu2-1*::pJD86 indicate the plasmids integrated into the *leu2-1* allele. Since pJD85 and pJD86 carry *LEU2*, these strains are Leu^+.

frameshift indicator unit, enzyme synthesis requires a frameshift event in a defined window within the L-A frameshift signal region. The other, the control, is identical except that it lacks the L-A frameshift signal and has β-galactosidase in the 0 frame so that its translation requires no frameshift. Assays of permeabilized yeast cells were as described previously (GUARENTE 1983). Cells were grown in H-trp or H-leu medium to the mid-logarithmic phase, and assays were normalized with respect to the optical density at 595 nm of the culture and to the assay time. Values are expressed as change in A_{420}/hr/OD_{595}. All assays were performed in triplicate.

Construction of frameshift indicator insertion strains: pJD85 and pJD86 were linearized with *Cla*I (in the *LEU2* gene), transformed into the *leu2-1* strain 10 and integrants were selected on H-leu medium. The integration of a single copy of the indicator units at *LEU2* was confirmed by Southern analysis and meiotic linkage (data not shown). These constructs have a non-functional *leu2-1* and a functional *LEU2* gene with the frameshift indicator unit in between.

Isolation of chromosomal mutations affecting ribosomal frameshifting: The isolation of the *mof1-1* (*hsh1-1*) mutation (strain EMS46) has been described (DINMAN and WICKNER 1992) and the *mof3-1* (strain EMS41) mutation was also isolated at the same time by the same methods. The *mof2-1*, *mof4-1*, *mof5-1*, *mof6-1*, *mof7-1*, and *mof8-1* mutations were generated from strain JD65-10B mutagenized with ethyl methanesulfonate (EMS) (LAWRENCE 1991). 8×10^4 cfu were seeded at a density of 1000 cfu/plate on H-leu containing 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) per ml, pH 6.8, and allowed to grow at 25°. A total of 206 blue colonies were initially assayed as permeabilized cells and 30 were identified as possible mutants, streaked for single colonies and assayed again. After two further rounds of assessment, 12 potential mutants remained.

Preparation of genomic DNA: Yeast cells (2.0 OD_{550} units) were converted to spheroplasts by digestion for 1 hr at 37° in 1 M sorbitol, 20 mM $KHPO_4$ (pH 6.5), 0.5 mM $CaCl_2$, 0.5% 2-mercaptoethanol, 10 μg/ml mureinase (U.S. Biochemical Corp.), harvested by centrifugation and resuspended in 10 mM Tris-Cl, pH 8.0, 1 mM EDTA (TE) containing 1% sodium dodecyl sulfate (SDS). An equal volume of phenol was added and the solutions were gently mixed at room temperature for 30 min. The aqueous phase was recovered by centrifugation and extracted in the same manner with phenol-chloroform and finally with chloroform. DNA was precipitated with ethanol and resuspended in 400 μl of TE. One fourth of each DNA preparation was digested with *Hin*dIII and RNAse A overnight at 37°, recovered by ethanol precipitation and resolved on a 1.0% Tris-acetate-EDTA agarose gel containing 0.5 μg of ethidium bromide per ml. DNA was transferred to nitrocellulose as recommended by the manufacturer (Schleicher & Schuell).

Preparation and analysis of dsRNA: dsRNA was prepared as described previously (FRIED and FINK 1978). Nucleic acids were resolved on 1.3% Tris-acetate-EDTA agarose gels containing 0.5 μg of ethidium bromide per ml.

RNA in the gels was denatured in two changes of 30 min each of 50% formamide, 9.25% formaldehyde, 1 × Tris-acetate-EDTA at room temperature and transferred to nitrocellulose in 20 × SSC as recommended by the manufacturer (Schleicher & Schuell).

Hybridizations: DNA-DNA hybridizations were carried out as follows. *Hin*dIII-digested pJD84 was labeled by random primer extension with [α-^{32}P]dATP according to the method of FEINBERG and VOLGELSTEIN (1983). Hybridizations followed the method of CHURCH and GILBERT (1984). Membranes were pre-hybridized for 5 min at 65° in hybridization buffer [1% bovine serum albumin (BSA), 1 mM disodium EDTA, 500 mM $NaHPO_4$, pH 7.2, 7% SDS, at 65°] and then hybridized with the probe overnight at 65°. The blots were washed with a buffer containing 0.5% BSA, 1 mM EDTA, 10 mM $NaHPO_4$, pH 7.2, 5% SDS for 10 min at 65°, and then in four changes of 1 mM EDTA, 10 mM $NaHPO_4$, pH 7.2, 1% SDS for 20 min each at 65°. The ionic strength of this solution is equivalent to 0.1 × SSC. Blots were then exposed to X-ray film.

RNA-RNA hybridizations were carried out as follows. L-A (−) strand RNA probe was made by T3 RNA polymerase run off transcripts of *Eco*RV-digested pLM1 (FUJIMURA and WICKNER 1992). M_1(−) strand probes were made by T3 RNA polymerase run off transcripts of *Pst*I-digested p596. The latter was made by inserting the 1.3-kb *Pst*I-*Sal*I fragment from pP-T_{316} (ZHU *et al.* 1993) into the Bluescript vector KS+ digested with the same enzymes. Membranes were pre-hybridized for 6 hr at 55° in 50% formamide, 5 × SSC, 50 mM $NaHPO_4$, pH 6.8, 0.1% SDS, 1 mM EDTA, 0.05% each of BSA, Ficoll, and polyvinylpyrrolidone and hybridized with probe in the same buffer at 55° overnight. Membranes were washed in five changes of 0.1 × SSC, 0.1% SDS at 65° for 20 min and exposed for autoradiography. L-A (−) strand probe was stripped from blots in two washes of 0.05 × SSC, 0.1% SDS at 95° for 10 min each.

RESULTS

Survey of known mutants for effects on frameshifting: Since the efficiency of frameshifting is related to the ability to maintain M_1 dsRNA (DINMAN and WICKNER 1992), we examined frameshifting in some known *mak* (*m*aintenance of *k*iller) mutants. A number of *SUP* mutants were also examined because of their ability to enhance translational misreading (WAKEM and SHERMAN 1990). We chose to examine *mod5* mutants, defective in isopentenyl modification of tRNAs (DIHANICH *et al.* 1987) because such modification may affect reading frame maintenance (HATFIELD and OROSZLAN 1990). The cytoplasmically inherited genetic element [ψ] (COX, TUITE and MCLAUGHLIN 1988) was examined because of its ability to enhance the suppression of frameshift mutations (CUMMINS *et al.* 1980). None of these mutations had any significant effect upon the efficiency of frameshifting (Table 2). While some of the parental strains did frameshift at efficiencies significantly different than the 1.9% generally observed in wild-type strains, genetic analysis showed that the different efficiencies of frameshifting were not due to the particular mutation in question, nor did they segregate as single genes in any of the meiotic tetrads tested.

TABLE 2

Survey of known mutants for effects on frameshifting

Genotype	Mutant/wild type	Method of determination[a]
SUP42	0.8	Assayed 6 tetrads from cross JD595[b]
SUP43	1.1	pF8/pTI25 ratios compared between SUP43 and sup$^+$ strains
SUP44	1.0	pF8/pTI25 ratios of 6 *SUP44* and 6 sup$^+$ spore clones
SUP45	0.9	pF8/pTI25 ratios of 6 *SUP45* and 6 sup$^+$ spore clones
SUP46	1.3	Assayed 6 tetrads from cross JD596 heterozygous for *SUP46*[b]
[ψ]	1.1	4 isogenic [ψ^+] and [ψ^-] strains (TUITE, MUNDY and COX 1981) assayed[b]
mod5		
+pF8	1	pF8/pTI25 ratios compared between isogenic *mod5* and *MOD5* strains
+pJD29	0.9	pJD29/pTI25 ratios compared between isogenic *mod5* and *MOD5* strains
+pJD32	0.8	pJD32/pTI25 ratios compared between isogenic *mod5* and *MOD5* strains
mak1	1.1	pF8/pTI25 ratios compared between *mak1*/*MAK1* isogenic strains
mak2-1	1.2	pF8/pTI25 ratios in a *mak2-1* strain
mak3-1	1.0	pF8/pTI25 ratios of 3 *mak3-1* and 3 *MAK*$^+$ spore clones
mak4-1	1.1	Assayed segregants of cross heterozygous for mak4-1[b]
mak6-1	1.2	pF8/pTI25 ratios in a *mak6-1* strain
mak7-1	0.7	pF8/pTI25 ratios in a *mak7-1* strain
mak8-1	1.0	Assayed segregants of cross heterozygous for *mak8-1*[b]
mak10	0.9	pF8/pTI25 ratios 3 *mak10* and 3 *MAK*$^+$ spore clones
mak12-1	1.1	Assayed segregants of cross heterozygous for *mak12-1*[b]
mak16-1	1.2	Assayed segregants of cross heterozygous for *mak16-1*[b]
mak18-1	1.0	Assayed segregants of cross heterozygous for *mak18-1*[b]
mak19-1	1.0	Assayed segregants of cross heterozygous for *mak19-1*[b]
mak20-1	1.3	pF8/pTI25 ratios in a *mak20-1* strain
mak24-1	0.7	Assayed segregants of cross heterozygous for *mak24-1*[b]
mak26-1	0.9	Assayed segregants of cross heterozygous for *mak26-1*[b]
pet18	1.0	pF8/pTI25 ratios in a *pet18* strain

[a] β-Galactosidase was measured either comparing the high copy frameshift plasmid pF8 with the high copy readthrough plasmid pTI25 or using the integrated frameshift indicator, pJD85 to compare mutant and wild type.

[b] Segregants with the integrated frameshift indicator (pJD85) were assayed.

Integration of frameshift indicator units into the *leu2-1* locus: We previously described isolation of a chromosomal mutation that affects the efficiency of −1 ribosomal frameshifting (DINMAN and WICKNER 1992). Analysis required the transformation of multiple meiotic segregants with both pF8 (the −1 ribosomal frameshift indicator plasmid) and pTI25 (the 0-

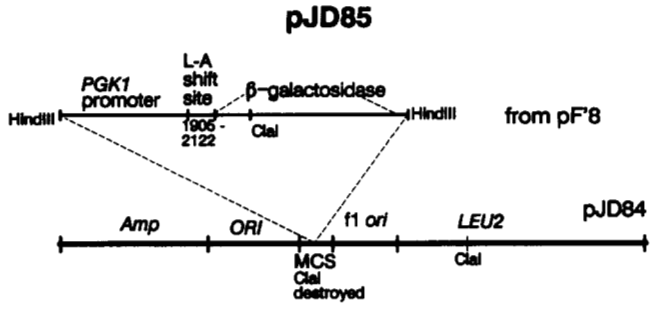

FIGURE 1.—Structure of pJD85, the integrating frameshift tester plasmid. The control plasmid, pJD86, lacks the L-A frameshift region and has β-galactosidase in frame with the first AUG, but otherwise is the same as pJD86 (see MATERIALS AND METHODS).

frame control plasmid). Two mutants were characterized using this method. To simplify screening and characterization, the β-galactosidase indicator units from pF8 and pTI25 were used to make the integrating frameshift (pJD85) and control (pJD86) plasmids which were integrated into the *leu2-1* locus of chromosome *III*. pJD85 contains (5′ to 3′) the yeast PGK1 promoter, a translational start codon, and the L-A frameshift site (bases 1905–2122) followed by the *E. coli lacZ* gene in the −1 frame with respect to the translational start site (Figure 1). pJD86 contains (5′ to 3′) the yeast *PGK1* promoter, a translational start codon, and the *E. coli lacZ* gene, which is in the 0 frame with respect to the translational start site.

The average β-galactosidase activity of the Leu$^+$ spore clones of cross JD63 (−1 ribosomal frameshift) was 0.18 and that of the Leu$^+$ spore clones of cross JD64 (0-frame control) was 8.4. This implies a frameshifting efficiency of 2.1%, comparable to the 1.9% frameshifting efficiencies reported for the episomal expression system of pF8 and pTI25.

Testing the integrated frameshift units using known frameshift mutants: The high frameshift mutants previously identified were crossed to strains containing the integrated −1 frameshifting unit and to strains containing the 0-frame control unit. In the former cross (JD67), heterozygous for *mof1* (referred to as *hsh1* in DINMAN and WICKNER, 1992), the segregants with the integrated frameshift segregated high:normal (0.30:0.09). In the 0-frame control cross (JD68), no significant differences were noted, and the average β-galactosidase activity was 4.9. Thus, the efficiency of −1 ribosomal frameshifting of *mof1* cells is 6.1% *vs.* 1.8% in the *MOF*$^+$ cells, *i.e.*, the *mof1*/*MOF*$^+$ ratio equals 3.4. These values are similar to the 3–3.5-fold increase in −1 ribosomal frameshift efficiency of *mof1* cells as determined from measurements based on the episomal plasmids (DINMAN and WICKNER 1992).

Similarly, when assayed using pF8 and pTI25, mof3 strains showed a 2.5–3-fold greater frameshift efficiency than wild-type cells. Using the integrated tester (cross JD76), frameshifting segregated 2 high (0.44 β-galactosidase units):2 normal (0.15 β-galactosidase units) and no significant differences were seen in the 0-frame controls (cross JD66, average of 7.7 β-galactosidase units). Thus, the wild-type showed 2.1% frameshifting efficiency and mof3 showed 5.7% or 2.9 times wild type. These experiments prove the utility of strains containing the integrated −1 frameshift indicator unit for the detection and genetic characterization of additional *mof* mutations.

Generation and genetic characterization of additional *mof*$^-$ strains: The single copy *lacZ* gene in the *MOF*$^+$ *leu2-1*::pJD85 strain JD65-10B, expressed at an efficiency of approximately 2%, confers a very light blue color to wild-type colonies. Therefore, our screen was best suited for the identification of cells that frameshift at higher than normal efficiencies. Potential *mof* mutants were chosen by their dark blue phenotypes on plates containing X-gal. Twelve Mof$^-$ candidates were crossed with the *MOF* strain JD348 and the diploids assayed. One had β-galactosidase activity typical of the Mof$^-$ phenotype, indicating that this mutation was dominant (data not shown). Since the diploids with JD348 failed to sporulate, to examine segregation of the Mof trait, the 11 remaining candidates were crossed to the *MOF* strain JD404, the diploid cells were sporulated and the resulting tetrads analyzed. In six of these crosses, β-galactosidase activities segregated 2 high:2 normal, indicative of a mutation in a single gene. The results of these six crosses plus the *mof1* and *mof3* results are summarized in Table 3. As all of the strains contain the *leu2-1*::pJD85 construct, results are expressed relative to wild-type. Mof$^-$ phenotypes ranged from an approximately 9-fold increase (cross JD477) to 2.7-fold increases (cross JD75). None of the mutant phenotypes were linked to mating type and thus to mutations in the integrated frameshift indicator units.

Spore clones from crosses JD75, 477, 65, 474, 468, 469, 471 and 472 were mated with each other and β-galactosidase activities were determined in the resulting diploids for complementation testing (Table 4). The eight mutations fall into eight different complementation classes. The β-galactosidase activities obtained from the diploids JD471 × JD65, JD477 × JD65 and JD474 × JD468 were intermediate between mutant and wild-type and so these diploids were sporulated. The resulting tetrads showed two gene segregation in each case, indicating that these represented different genes. These genes were designated *mof1-1* to *mof8-1*.

***mof2-1*, *mof5-1* and *mof6-1* mutants are ts for growth with cell-cycle arrest phenotypes:** In general we noted that the greater the increase in −1 ribosomal frameshifting, the less healthy the cells. *mof1-1*, *mof2-1*, *mof4-1* and *mof5-1* cells formed smaller colo-

TABLE 3

Meiotic segregation of *mof*

mof	Cross	Frameshift efficiency (× wild-type)[a]	No. of tetrads analyzed	Segregation[b] A	B	C	D	Mean ± σ wild-type segregants	Mean ± σ *mof* segregants
+	Wild-type	1	12	0.13	0.09	0.10	0.14		
1	JD75	2.7	8	0.65	0.26	0.42	0.16	0.24 ± 0.07	0.64 ± 0.14
				0.53	0.20	0.12	0.52		
				0.42	0.26	0.65	0.25		
2	JD477	8.9	9	0.86	0.95	0.10	0.07	0.12 ± 0.03	1.07 ± 0.33
				1.00	1.30	0.09	0.10		
				0.81	0.81	0.15	0.21		
3	JD76	2.8	23	0.20	0.43	0.12	0.29	0.16 ± 0.04	0.45 ± 0.11
				0.13	0.32	0.16	0.71		
				0.17	0.37	0.19	0.48		
4	JD474	4.4	12	0.09	0.07	0.32	0.38	0.11 ± 0.03	0.49 ± 0.16
				0.08	0.10	0.43	0.72		
				0.89	0.52	0.11	0.27		
5	JD468	3.5	10	1.00	1.10	0.36	0.22	0.23 ± 0.06	0.82 ± 0.21
				1.16	0.26	0.75	0.26		
				0.83	0.66	0.15	0.23		
6	JD469	3.3	9	0.16	0.11	0.28	0.31	0.10 ± 0.02	0.33 ± 0.04
				0.10	0.14	0.34	0.24		
				0.26	0.14	0.36	0.14		
7	JD471	2.9	9	0.37	0.25	0.14	0.09	0.12 ± 0.03	0.35 ± 0.05
				0.34	0.30	0.11	0.11		
				0.09	0.16	0.35	0.35		
8	JD472	3.3	6	0.41	0.17	0.17	0.57	0.15 ± 0.03	0.50 ± 0.09
				0.64	0.18	0.40	0.15		
				0.51	0.13	0.19	0.40		

[a] Average of *mof* segregants.

[b] β-Galactosidase activities are shown from typical tetrads in each cross. Each value is the average of three determinations. The OD_{595} measurements were done in the linear range. We compared measurements of the specific activities of a series of wild-type and *mof* mutants, either as described here (β-galactosidase activity of whole cells/OD_{595}) or as β-galactosidase activity of crude extracts/protein. The results were almost identical.

TABLE 4

Complementation tests

Segregants *mof* from cross		*mof* *1* JD75	*2* JD477	*3* JD65	*4* JD474	*5* JD468	*6* JD469	*7* JD471	*8* JD472	+ WT
1	JD75	0.59	0.25	0.25	0.27	0.29	0.29	0.20	0.20	0.18
2	JD477		1.33	0.38	0.28	0.27	0.23	0.20	0.14	0.32
3	JD65			0.54	0.28	0.18	0.16	0.32	0.22	0.18
4	JD474				0.76	0.32	0.19	0.22	0.15	0.26
5	JD468					0.75	0.25	0.26	0.17	0.25
6	JD469						0.41	0.14	0.17	0.17
7	JD471							0.36	0.18	0.15
8	JD472								0.36	0.12

nies than wild-type cells. In cross JD477, all of the 18 *mof2-1* spore clones from nine complete tetrads were temperature sensitive for growth at 8° and at 37°, and they all grew poorly at 30° on a non-fermentable carbon source (glycerol). In cross JD468, Mof$^-$ (*mof5-1*), ts (37°) and cs (8°) growth co-segregated in 10 tetrads. In cross JD469, *mof6-1* and temperature sensitivity at 37° cosegregated in all 6 tetrads. Temperature sensitive cells of strains JD477-1A (*mof2-1*), JD468-2A (*mof5-1*) and JD469-3A (*mof6-1*) cells were grown to mid-log phase at permissive temperature (30°) and approximately 8×10^7 cells were plated on H-leu and grown at 37°. Three temperature-resistant revertants were obtained from each and β-galactosidase activities were measured (Table 5). Reversion to growth at 37° resulted in the partial reversion of *mof2-1* and *mof5-1* and in the total reversion to wild-type of *mof6-1* cells.

When shifted to 37° for 14 h, *mof2-1* cells had a uniform dumbell appearance (Figure 2). The *mof5-1* mutants showed a multiple bud arrest phenotype, and the *mof6-1* strains appeared as single large unbudded cells. All three mutants complemented each other as well as mutations in *cdc1*, *3* to *12*, *14* to *19*, *21*, *23*, *24*, *25*, *27* to *31*, *33* to *37*, *39*, *60*, *63*, *64*, and *65*.

Ability of *mof*$^-$ cells to maintain the L-A and M_1 viruses: L-A and M_1 were cytoduced into *mof* and *MOF* cells at 30° and at 20° (Table 6). When cyto-

TABLE 5

Co-reversion of ts growth and Mof phenotype

Mutation	Strain	β-Galactosidase (OD_{420}/hr/OD_{595})			
		ts strain	tr revertant 1	tr revertant 2	tr revertant 3
mof2-1	477-1A	0.73	0.40	0.34	0.42
mof5-1	468-2A	0.87	0.27	0.27	0.32
mof6-1	469-3A	0.37	0.15	0.12	0.12

Mutation	Strain	ts mutant		tr revertant 1	
		L-A-HN M_1	L-A-HNB M_1	L-A-HN M_1	L-A-HNB M_1
mof2-1	477-1A	−	−	+	+
mof5-1	468-2A	−	−	−	+
mof6-1	469-3A	−	−	+	+

Cytoduction donors were strains 1074 and 2631 (see legend for Table 6). − = M_1 not maintained; + = M_1 maintained in cytoductants. ts, temperature sensitive; tr, temperature resistant.

ductions were done at 30°, only *mof3-1*, *mof7-1* and *mof8-1* cells were killers. *mof1-1*, *mof2-1*, *mof4-1*, *mof5-1* and *mof6-1* strains were non-killers whether the cytoduction donor had L-A-HN or L-A-HNB, two natural variants of the L-A virus (UEMURA and WICKNER 1988). When cytoductions were performed at 20°, only *mof6-1* cells were non-killers; the remainder had the killer phenotype, although such *mof1*, *mof2* and *mof5* killer cytoductants lost M_1 when grown to single colonies at 30° or 32° (Table 6). The Mof$^-$ and loss of M_1 phenotypes of *mof1-1* cosegrated in 6 tetrads. The Mof$^-$ loss of M_1, and ts growth phenotypes of *mof2-1*, *mof5-1* and *mof6-1* cosegregated in 5, 6 and 9 tetrads, respectively. The temperature-resistant revertants also reverted for ability to maintain M_1, although the *mof5* partial revertant was still unable to maintain M_1 in the presence of L-A-HN (Table 5). These results indicate that the ts and loss of M_1 phenotypes are due to the same mutations as the mof phenotypes.

Northern blots of total nucleic acid extracts of the cytoductants were probed for M_1 (Figure 3). These affirm the killer assay data, *i.e.*, that M_1 dsRNA was present in all of the killer strains. Low levels of M_1 dsRNA detected in *mof5* cells cytoduced at 30° with L-A-HNB disappeared on subcloning. Also, in *mof5* cells cytoduced at 20°, there are a series of discrete bands with greater electrophoretic mobility which hybridize with the M_1 (−) strand probe, perhaps indicative of deletion mutants. Such a band is also seen in *mof8* mutants cytoduced at 20°. All of the *mof* mutants were able to maintain L-A, and L-A copy number generally increased if M_1 was lost (data not shown) as previously observed (BALL, TIRTIAUX and WICKNER 1984).

To test for an effect of the *mof1-1*, *mof2-1*, *mof4-1*, *mof5-1*, *mof6-1* or *mof8-1* mutations on L-A copy number, we carried out crosses with *MOF* strains in which neither parent had M_1. RNA extracted in equal amounts from each segregant of each of 4 tetrads of each cross was analyzed by agarose gel electrophoresis and ethidium staining. No effect of any of these mutations on L-A copy number was observed.

We also examined the effect of *mof6-1* on propagation of X dsRNA, a deletion mutant of L-A lacking nearly the entire L-A coding region, but carrying the packaging, replication and transcription signals of L-A (ESTEBAN and WICKNER 1988; ESTEBAN, FUJIMURA and WICKNER 1988). Cytoduction of X and L-A-HNB into each of six *mof6-1* meiotic segregants produced cytoductants all of which lost X dsRNA, while two *MOF* segregants maintained X.

Temperature-dependent frameshifting in *mof* mutants: The temperature-dependent maintenance of M_1 and the ts growth phenotype of several *mof* mutants led us to determine whether the frameshifting efficiency was temperature dependent. We found that frameshifting at 30°/frameshifting at 20° for *MOF*, *mof1-1*, *mof2-1*, *mof4-1*, *mof5-1* and *mof6-1* were 1.0, 1.8, 3.8, 1.5, 2.1 and 2.1, respectively. Thus, at 20°, the mutants were only marginally increased in frameshifting efficiency, and this presumably explains why M_1 was stably maintained in most of the mutants at 20°. Although comparable measurements of frameshift efficiency at 37° could not be done in strains that do not grow at that temperature, it is likely that frameshifting efficiency is even higher at this temperature, explaining the growth defects.

Effects of *mof* mutations upon frameshifting efficiencies of different (plasmid based) slippery sites: Wild-type and *mof* cells were transformed with the high copy control and frameshift indicator plasmids, and with high copy plasmids containing different slippery sites. β-Galactosidase activities were measured in cells with and without plasmids and the data were corrected for the low amount of β-galactosidase activity produced by the single integrated unit alone. The results with pF8 were compared to 0-frame controls (pTI25) to give frameshift efficiencies. *mof*$^-$/wild-type ratios were also compared. The results are shown in Table 7.

The *mof1-1* mutation produces substantial absolute changes when the P site contains C or G residues (*e.g.*, pF8, pJD31, pJD24, pJD26, pJD30), with correspondingly small or negative effects with A or T in the P-site (*e.g.*, pJD13, pJD32). In the case of *mof2-1*, there are big absolute and relative increases in all slippery sites, but no change when the pseudoknot is disrupted (pJD18). *mof3-1* shows a general loss of frame control, but the greatest effects are observed when the A-site contains G or C residues (*e.g.*, pJD26 and pJD28). With *mof4-1*, the greatest increase (24X) occurred with the slippery site of pJD23 (GGGTAAA). A GGT codon in the 0-frame P-site (pF8, pJD11, pJD23,

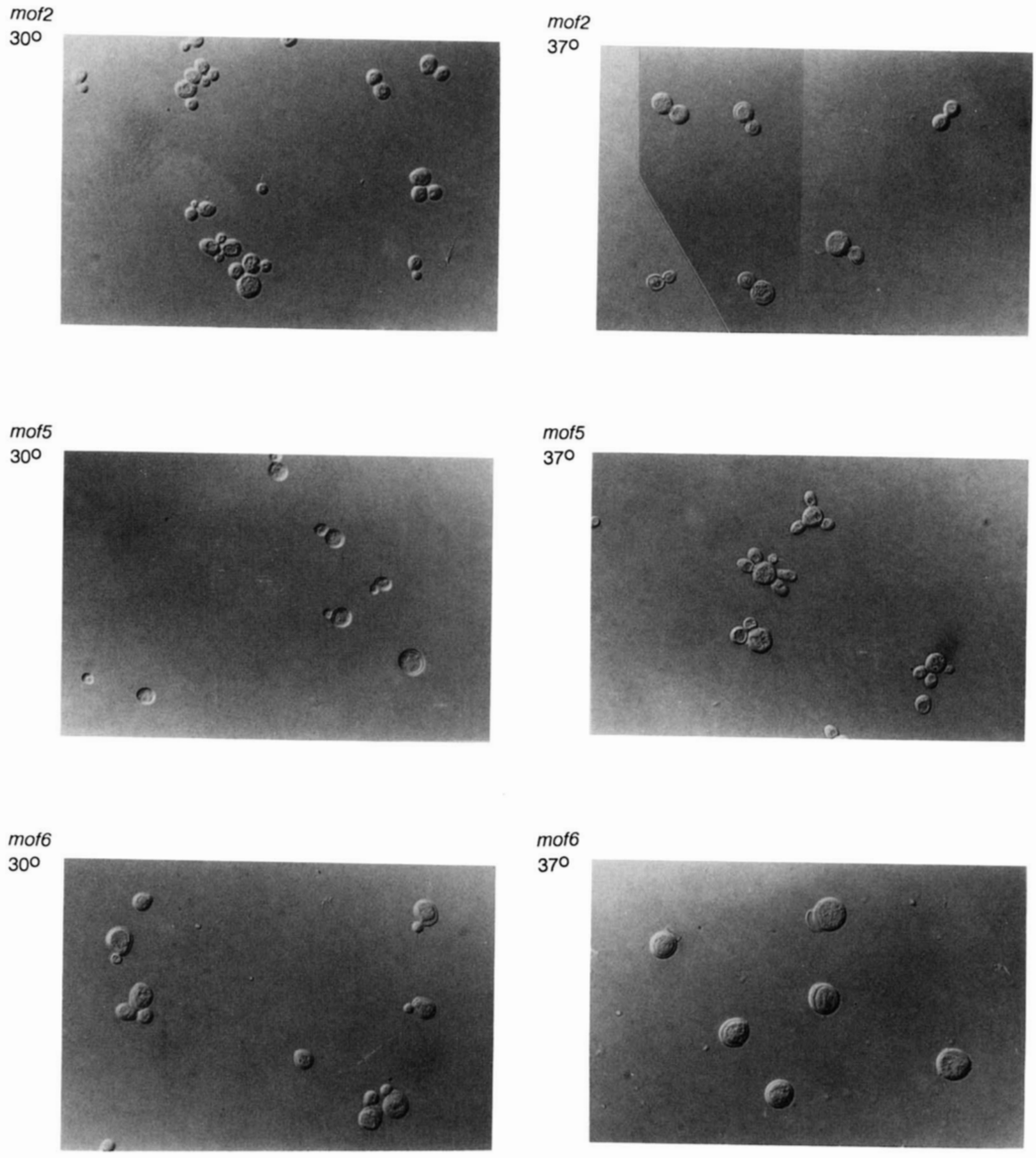

FIGURE 2.—Arrest phenotypes of *mof* mutants. Cells growing in H-leu at 30° or 14 hr after a shift to 37° were fixed with 3.5% formaldehyde in phosphate buffered saline and photographed at 100×.

pJD30) appears to maximize the effect of *mof5-1*. There are no clearly discernable patterns for *mof6-1* and *mof7-1*.

DISCUSSION

The importance of studying maintenance of reading frame in translation is enhanced by the manipulation of this process by various RNA viruses and retroviruses. The efficiency with which these frameshift events occur varies dramatically among systems, probably reflecting the requirement that the ratio of the normally terminated protein (*e.g.*, Gag) to the fusion protein (*e.g.*, Gag-Pol) be optimal for viral propagation. The ability of the L-A dsRNA virus to support the M_1 satellite dsRNA replicon has been shown to require a gag/gag-pol ratio within 2-fold of the normal ratio of about 60/1 (DINMAN and WICKNER 1992). Decreased frameshifting by the yeast retrovirus (retrotransposon) Ty*1* has likewise been shown to produce decreased transposition (XU and BOEKE 1990). We argued that the ratio of the L-A viral proteins must be critical for the assembly process. There are estimated to be 120 Gag molecules per particle (ESTEBAN and WICKNER 1986), and symmetry requirements suggest that the particles are made up of 60 Gag dimers. The normal frameshift efficiency (1.9%) suggests there are 2 Gag-Pol molecules per particle. We suggest that these form a dimer, and that this dimer binds viral (+) strands to initiate packaging and particle assembly.

Do *mof* mutations primarily affect −1 ribosomal frameshifting? In this study we have characterized eight mutants in eight different genes affecting the efficiency with which β-galactosidase is made from a construct that requires a −1 shift of frame after the

TABLE 6

Cytoduction of L-A and M_1 into *mof* mutants

Mutant	Maintenance (+) or loss (−) of M_1 dsRNA		
	Cytoduction at 20°	Cytoduction at 30°	M_1^+ 20° cytoductants restreaked at 30° or 32°
mof1	+	−	−
mof2	+	−	−
mof3	+	+	
mof4	+	−	+
mof5	+	−	−
mof6	−	−	
mof7	+	+	
mof8	+	+	

Identical results were obtained with cytoduction donors carrying L-A-HN and L-A-HNB.

Cytoduction donor strains

1074: *MAT***a** *leu1 kar1-1* L-AHN$_{A364A}$ M_1
2631: *MAT***a** *leu1 kar1-1* L-AHNB M_1
RE406: *MAT***a** *leu1 kar1-1* L-A-HNB X

Cytoduction acceptor strains

JD599: *MATα leu2-1*::pJD85 *his4 trp1 mof1* rho°
JD609: *MATα leu2-1*::pJD85 *ura3 his4 trp1 ade2 mof2* rho°
JD610: *MATα leu2-1*::pJD85 *ura3* rho°
JD600: *MATα leu2-1*::pJD85 *his4 trp1 ade2 ura3 mof3* rho°
JD633: *MATα leu2-1*::pJD85 *ura3 his4 trp1 ade2 mof4* rho°
JD601: *MATα leu2-1*::pJD85 *ura3 his4 trp1 ade2 mof5* rho°
JD602: *MATα leu2-1*::pJD85 *ura3 his4 trp1* rho°
JD603: *MATα leu2-1*::pJD85 *ura3 trp1 ade2 mof6* rho°
JD604: *MATα leu2-1*::pJD85 *ura3 ade2* rho°
JD605: *MATα leu2-1*::pJD85 *ura3 his4 trp1 mof7* rho°
JD606: *MATα leu2-1*::pJD85 *ura3* rho°
JD607: *MATα leu2-1*::pJD85 *ura3 ade2 mof8* rho°
JD608: *MATα leu2-1*::pJD85 *ura3 his4 ade2* rho°

translational start. That these mutations are not *SUF* mutants in tRNA genes (reviewed by HINNEBUSCH and LEIBMAN 1991) is indicated by the fact that all are recessive, unlike almost all tRNA mutations which are dominant. Moreover, our detailed studies of the effects of particular *mof* mutations on specific slippery sites (Table 7) show that their effects depend critically on this small seven nucleotide sequence. This is detailed here for each mutant studied:

- *mof1-1* increases shifting 2.2× at the normal site GGGTTTA, but has no effect on the site AAATTTA and decreases shifting at TTTTTTA.
- *mof2-1* increases shifting from 11.4% (wild type host) to 22.4% at the site TTTTTTA, a change of 11%, but only increases shifting from 0.37% to 0.9% at the site GAATTTA, a change of only 0.5%. Moreover, it has no effect on a slippery signal whose pseudoknot has been destroyed (pJD18).
- *mof3-1* increases frameshifting from 11.4% to 24% at the site TTTTTTA, but has no effect on the site GAATTTA.
- *mof4-1* increases frameshifting from 11.4% to 21.3% at the site TTTTTTA, but only from 0.1% to 0.2% at the site AGGTTTA.
- *mof5-1* increases frameshifting 2.5-fold from 2.1% to 5.2% at the normal site GGGTTTA, but has no effect on the site CCCTTTT and decreases shifting at TTTTTTA.
- *mof6-1* increases frameshifting 2.4-fold from 2.1% to 5.0% at the normal site GGGTTTA but has no effect at GAATTTA or TTTGGGC.
- *mof7-1* increases frameshifting 1.6-fold at the normal site GGGTTTA, but has no effect at the site GAATTTA.

Thus, each of the mutants examined (*mof8-1* has not been studied in this regard) affects ribosomal frameshifting within the L-A mRNA segment responsible for this process (Table 8).

Pleiotropic phenotypes of *mof* mutants: Most of these mutants were unable to maintain the M_1 satellite dsRNA, as expected from our earlier results. Although the assembly process for M_1 and L-A is believed to be essentially the same, we were surprised to find that the L-A genome is not lost by these mutants. In fact, its copy number is not even reduced in *mof* strains. To determine if the difference between L-A and M_1 is due to differences between the L-A and M_1 signals for replication, packaging or transcription, we examined the effect of the *mof6-1* mutation on propagation of X dsRNA, a deletion mutant of L-A retaining only 530 bp of L-A's 4.6 kb (ESTEBAN and WICKNER 1988; ESTEBAN, FUJIMURA and WICKNER 1988). X, like M_1, was lost from *mof6-1* strains. How can we explain this difference? The similarity of M_1 and X in this regard suggests that it is L-A's encoding the viral coat proteins that makes it different from M_1 and X. For this to be important would require that *cis*-packaging is occurring in this system. That is, coat proteins made from a particular L-A mRNA molecule preferentially package their own message rather than another L-A or M_1 or X (+) ssRNA. When frameshifting is more efficient than normal, more Gag-Pol proteins are made than usual, and for M_1 or X, which must get Gag-Pol and Gag from the free pool of proteins, Gag will often not be available in sufficient quantity. But an L-A (+) ssRNA molecule that has been bound at the packaging site inside *pol* by a Gag-Pol dimer, is its own captive source of further Gag protein. The 5′ end and *gag* open reading frame of this L-A (+) strand are available to ribosomes until the closure of the viral particle shuts them out. The notion of *cis*-packaging has also been suggested to explain results of studies of exclusion of L-A by expression of Gag and Gag-Pol from full-length cDNA clones of L-A (VALLE and WICKNER 1993).

We anticipate that most of the *mof* genes encode components of the translation apparatus involved in elongation. The striking cell cycle and Pet$^-$ phenotypes of *mof2-1*, *mof5-1* and *mof6-1* may well be indirect, resulting from either the production of abnormal proteins due to frameshifts in places where they do

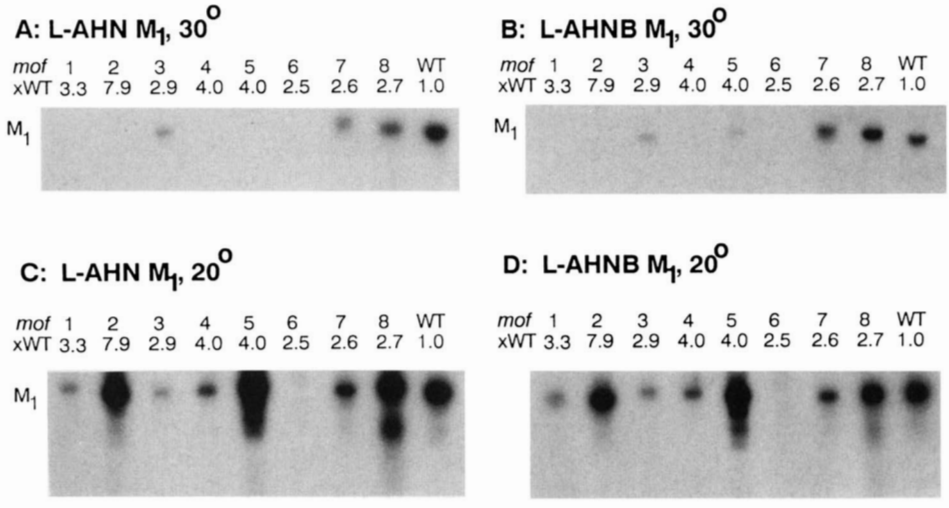

FIGURE 3.—M_1 is lost from *mof1*, *mof2*, *mof4*, *mof5* and *mof6* mutants. Nucleic acids were extracted from cytoductants from 30° cytoductions (Table 6), analyzed by 1.3% agarose gel electrophoresis, blotted to nitrocellulose and probed with an M_1 probe as described in MATERIALS AND METHODS.

TABLE 7

***mof* mutations differentially affect specific slippery sites**

Plasmid	Slip site	WT	*mof1*	*mof2*	*mof3*	*mof4*	*mof5*	*mof6*	*mof7*
Functional slippery sites									
pF8	GGGTTTA	2.1%	4.7%	9.5%	3.3%	5.1%	5.2%	5.0%	3.4%
			2.2x	4.5x	1.6x	2.4x	2.5x	2.4x	1.6x
pJD13	AAATTTA	2.5%	2.7%	6.6%	4.9%	5.1%	4.7%	3.1%	2.2%
			1.1x	2.7x	2.0x	2.0x	1.9x	1.2x	0.9x
pJD31	CCCTTTT	4.4%	9.1%	8.2%	8.4%	13.3%	4.5%	6.1%	3.1%
			2.1x	1.9x	1.9x	3x	1x	1.4x	0.7x
pJD24	GGGAAAA	4.8%	9.4%	9.8%	8.7%	16%	3.4%	9.6%	3.2%
			2x	2x	1.8x	3.3x	0.7x	2x	0.7x
pJD32	TTTTTTA	11.4%	7.7%	22.4%	23.9%	21.3%	6.7%	19.2%	13.6%
			0.7x	2.6x	2.1x	1.9x	0.6x	1.7x	1.2x
P-Site: non-functional									
pJD11	AGGTTTA	0.1%	0.56%	0.9%	0.6%	0.2%	0.9%	0.9%	0.1%
			5.6x	9x	6x	2x	9x	9x	1x
pJD12	GAATTTA	0.37%	0.9%	0.9%	0.3%	0.7%	0.7%	0.2%	0.2%
			2.4x	2.4x	1x	1.9x	1.9x	0.5x	0.5x
A-Site: non-functional									
pJD23	GGGTAAA	0.15%	0.55%	1.4%	0.9%	3.6%	1.3%	0.6%	0.4%
			3.7x	9.3x	6x	24x	8.7x	4x	2.6x
pJD26	GGGCCCC	0.4%	2.5%	3.0%	3.4%	1.3%	0.9%	0.8%	0.8%
			6.3x	7.5x	8.5x	3.2x	2.3x	2x	2x
pJD28	TTTGGGC	0.13%	0.7%	1.2%	1.2%	1%	0.6%	0.1%	0.3%
			5.4x	9.2x	9.2x	7.7x	4.6x	1x	2.3x
pJD30	GGGTTTG	0.31%	2.8%	2.0%	1.3%	2.2%	2.1%	0.4%	0.7%
			9.0x	6.5x	4.2x	7.1x	6.8x	1.3x	2.3x
Pseudoknot mutation									
pJD18	5′cPsi	0.1%	0.16%	0.1%	0.4%	0.8%	0.5%	0.2%	0.1%
			1.6x	1x	4x	8x	5x	2x	1x
TY*1* slippery sites									
pJD58	TY − codon	0.49%	0.84%	3.4%	3.7%	ND	2.1%	0.94%	1.7%
			1.7x	6.9x	7.6x		4.2x	1.9x	3.5x
pJD70	TY + codon	1.7%	3.7%	2.2%	2.8%	ND	3.2%	1.3%	1.8%
			2.1x	1.3x	1.6x		1.9x	0.8x	1.1x

Wild-type cells were JD63-3D; *mof1* cells were JD67-1C; *mof2* cells were JD477-3A; *mof3* cells were JD65-10C; *mof4* cells were JD474-5A; *mof5* cells were JD468-2B; *mof6* cells were JD469-5C; *mof7* cells were JD471-6D. "%" values are β-galactosidase activity relative to the 0 frame plasmid, pTI25. "x" values show the frameshifting of mutant/wild-type. "Ty − codon" means without an extra codon between the +1 shift site and the pseudoknot, while "Ty + codon" means with the extra codon (DINMAN and WICKNER 1992). Sample statistics: *mof2-1* host with pJD32 shows 22.4% frameshifting with $\sigma_{n-1} = 2.9\%$. A *mof5-1* host with pF′8 shows 5.2% frameshifting with $\sigma_{n-1} = 0.28\%$. A *MOF*$^+$ host with pJD23 shows 0.15% frameshifting with $\sigma_{n-1} = 0.01\%$.

TABLE 8

Summary of properties of *mof* mutants

Mutant	Frameshifting (fold wild type)	M_1 dsRNA	ts	Arrest phenotype	Pet
mof1-1	2.7	−	+		+
mof2-1	8.9	−	ts	Dumbell	Pet
mof3-1	2.8	+	+		+
mof4-1	4.4	−	+		+
mof5-1	3.5	−	ts	Multi-bud	Pet
mof6-1	3.3	−	ts	Large, unbudded	+
mof7-1	2.9	+	+		+
mof8-1	3.3	+	+		+

The fold increase in frameshifting efficiency here is from the data in Table 3. ts, temperature sensitive.

not normally occur, or abnormal amounts of cellular proteins made by frameshifting. It is striking, however, that no eukaryotic cellular genes are known to require ribosomal frameshifting for their expression. These cell cycle phenotypes will require further detailed study.

The specificity of the various *mof* mutants for particular slippery sites suggests that these studies will be useful in examining the function of different components of the translation apparatus. While the results presented here are still preliminary, we have clearly shown that particular *mof* mutations affect different slippery sites differently. The *mof1-1* mutation seems to preferentially affect slippery sites in which the P site had G or C residues, while the *mof3-1* mutation shows its greatest effects on slippery sites with G's or C's in the A site. Such functional specificities, in conjunction with structural information about the *MOF* gene products should facilitate dissection of the role of individual proteins in the process of translation elongation.

There are no known eukaryotic cellular genes that require ribosomal frameshifting for their expression. The fact that mutations affecting M_1 viral propagation are tolerated by the cell supports our earlier suggestion that drugs altering ribosomal frameshifting may be useful as antiretrovirals. In particular, the *MOF* products are potential targets for such drugs.

We thank ROLF STERNGLANZ for *top1* (*mak1*) disruption strains, ANITA HOPPER for *mod5* disruption strains, and PAUL WAKEM and FRED SHERMAN for *SUP42*, *SUP43*, *SUP44*, *SUP45* and *SUP46* strains.

LITERATURE CITED

ATKINS, J. F., R. B. WEISS, S. THOMPSON and R. F. GESTELAND, 1991 Towards a genetic dissection of the basis of triplet decoding, and its natural subversion: programmed reading frame shifts and hops. Annu. Rev. Genet. **25:** 201–228.

BALL, S. G., C. TIRTIAUX and R. B. WICKNER, 1984 Genetic control of L-A and L-BC dsRNA copy number in killer systems of *Saccharomyces cerevisiae*. Genetics **107:** 199–217.

BELCOURT, M. F., and P. J. FARABAUGH, 1990 Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. Cell **62:** 339–352.

BRIERLEY, I., P. DINGARD and S. C. INGLIS, 1989 Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. Cell **57:** 537–547.

BRIERLEY, I., A. J. JENNER and S. C. INGLIS, 1992 Mutational analysis of the "slippery-sequence" component of a coronavirus ribosomal frameshifting signal. J. Mol. Biol. **227:** 463–479.

BRIERLEY, I., N. J. ROLLEY, A. J. JENNER and S. C. INGLIS, 1991 Mutational analysis of the RNA pseudoknot component of a coronavirus ribosomal frameshifting signal. J. Mol. Biol. **220:** 889–902.

CHANDLER, M., and O. FAYET, 1993 Translational frameshifting in the control of transposition in bacteria. Mol. Microbiol. **7:** 497–503.

CHURCH, G. M., and W. GILBERT, 1984 Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:** 1991–1995.

COX, B. S., M. F. TUITE and C. S. MCLAUGHLIN, 1988 The Psi factor of yeast: a problem in inheritance. Yeast **4:** 159–179.

CUMMINS, C. M., R. F. GABER, M. R. CULBERTSON, R. MANN and G. R. FINK, 1980 Frameshift suppression in *Saccharomyces cerevisiae*. III. Isolation and genetic properties of group III suppressors. Genetics **95:** 855–875.

DIHANICH, M. E., D. NAJARIAN, R. CLARK, E. C. GILLMAN, N. C. MARTIN and A. K. HOPPER, 1987 Isolation and characterization of MOD5, a gene required for isopentenylation of cytoplasmic and mitochondrial tRNAs of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 177–184.

DINMAN, J. D., T. ICHO and R. B. WICKNER, 1991 A-1 ribosomal frameshift in a double-stranded RNA virus of yeast forms a gag-pol fusion protein. Proc. Natl. Acad. Sci. USA **88:** 174–178.

DINMAN, J. D., and R. B. WICKNER, 1992 Ribosomal frameshifting efficiency and *gag/gag-pol* ratio are critical for yeast M_1 double-stranded RNA virus propagation. J. Virol. **66:** 3669–3676.

ENG, W. K., S. D. PANDIT and R. STERNGLANZ, 1989 Mapping of the active site tyrosine of eukaryotic DNA topoisomerase I. J. Biol. Chem. **264:** 13373–13376.

ESTEBAN, R., T. FUJIMURA and R. B. WICKNER, 1988 Site-specific binding of viral plus single-stranded RNA to replicase-containing open virus-like particles of yeast. Proc. Natl. Acad. Sci. USA **85:** 4411–4415.

ESTEBAN, R., and R. B. WICKNER, 1986 Three different M1 RNA-containing viruslike particle types in *Saccharomyces cerevisiae*: in vitro M1 double-stranded RNA synthesis. Mol. Cell. Biol. **6:** 1552–1561.

ESTEBAN, R., and R. B. WICKNER, 1988 A deletion mutant of L-A double-stranded RNA replicates like M1 double-stranded RNA. J. Virol. **62:** 1278–1285.

FRIED, H. M., and G. R. FINK, 1978 Electron microscopic heteroduplex analysis of "killer" double-stranded RNA species from yeast. Proc. Natl. Acad. Sci. USA **75:** 4224–4228.

FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

FUJIMURA, T., and R. B. WICKNER, 1988 Gene overlap results in a viral protein having an RNA binding domain and a major coat protein domain. Cell **55:** 663–671.

FUJIMURA, T., and R. B. WICKNER, 1992 Interaction of two cis sites with the RNA replicase of the yeast L-A virus. J. Biol. Chem. **267:** 2708–2713.

FUJIMURA, T., J. C. RIBAS, A. M. MAKHOV and R. B. WICKNER, 1992 Pol of gag-pol fusion protein required for encapsidation of viral RNA of yeast L-A virus. Nature **359:** 746–749.

GUARENTE, L., 1983 Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. Methods Enzymol. **101:** 181–191.

HATFIELD, D., and S. OROSZLAN, 1990 The where, what and how

of ribosomal frameshifting in retroviral protein synthesis. Trends Biochem. Sci. **15:** 186–190.

HATFIELD, D., J. G. LEVIN, A. REIN and S. OROSZLAN, 1992 Translational suppression in retroviral gene expression. Adv. Virus Res. **41:** 193–239.

HINNEBUSCH, A. G., and S. W. LEIBMAN, 1991 Protein synthesis and translational control in *Saccharomyces cerevisiae*, pp. 627–735, in *The Molecular Biology of the Yeast Saccharomyces*, edited by J. R. BROACH, J. R. PRINGLE and E. W. JONES. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

HOPPER, J. E., K. A. BOSTIAN, L. B. ROWE and D. J. TIPPER, 1977 Translation of the L-species dsRNA genome of the killer-associated virus-like particles of *Saccharomyces cerevisiae*. J. Biol. Chem. **252:** 9010–9017.

ICHO, T., and R. B. WICKNER, 1989 The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. J. Biol. Chem. **264:** 6716–6723.

ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JACKS, T., 1990 Translational suppression in gene expression in retroviruses and retrotransposons. Curr. Top. Microbiol. Immunol. **157:** 93–124.

JACKS, T., H. D. MADHANI, F. R. MASIARZ and H. E. VARMUS, 1988 Signals for ribosomal frameshifting in the Rous sarcoma virus gag-pol region. Cell **55:** 447–458.

LAWRENCE, C. W., 1991 Classical mutagenesis techniques, pp. 273–281, in *Guide to Yeast Genetics and Molecular Biology*, edited by C. GUTHRIE and G. R. FINK. Academic Press, New York.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*, Ed. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

RIBAS, J. C., and R. B. WICKNER, 1992 RNA-dependent RNA polymerase consensus sequence of the L-A double-stranded RNA virus: definition of essential domains. Proc. Natl. Acad. Sci. USA **89:** 2185–2189.

RIDLEY, S. P., S. S. SOMMER and R. B. WICKNER, 1984 Superkiller mutations in *Saccharomyces cerevisiae* suppress exclusion of M2 double-stranded RNA by L-A-HN and confer cold sensitivity in the presence of M and L-A-HN. Mol. Cell. Biol. **4:** 761–770.

SHERMAN, F., 1991 In: C. GUTHRIE, G. R. FINK, ed. Guide to yeast genetics and molecular biology. Methods Enzymol. **194:** 3–21.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

TEN DAM, E., K. PLEIJ and D. DRAPER, 1992 Structural and functional aspects of RNA pseudoknots. Biochemistry **31:** 11665–11676.

TU, C., T.-H. TZENG and J. A. BRUENN, 1992 Ribosomal movement impeded at a pseudoknot required for ribosomal frameshifting. Proc. Natl. Acad. Sci. USA **89:** 8636–8640.

TUITE, M. F., C. R. MUNDY and B. S. COX, 1981 Agents that cause a high frequency of genetic change from [psi+] to [psi−] in *Saccharomyces cerevisiae*. Genetics **98:** 691–711.

UEMURA, H., and R. B. WICKNER, 1988 Suppression of chromosomal mutations affecting M_1 virus replication in *Saccharomyces cerevisiae* by a variant of a viral RNA segment (L-A) that encodes coat protein. Mol. Cell. Biol. **8:** 938–944.

VALLE, R. P. C., and R. B. WICKNER, 1993 Elimination of L-A dsRNA virus of yeast by expression of *gag* and *gag-pol* from an L-A cDNA clone. J. Virol. **67:** 2764–2771.

WAKEM, L. P., and F. SHERMAN, 1990 Isolation and characterization of omnipotent suppressors in the yeast *Saccharomyces cerevisiae*. Genetics **124:** 515–522.

WICKNER, R. B., 1991 Methods in classical genetics, pp. 101–147, in *Saccharomyces*, edited by M. F. TUITE and S. G. OLIVER. Plenum Press, New York.

WICKNER, R. B., and M. J. LEIBOWITZ, 1976 Two chromosomal genes required for killing expression in killer strains of *Saccharomyces cerevisiae*. Genetics **82:** 429–442.

XU, H., and J. D. BOEKE, 1990 Host genes that influence transposition in yeast: the abundance of a rare tRNA regulates Ty*1* transposition frequency. Proc. Natl. Acad. Sci. USA **87:** 8360–8364.

ZHU, Y.-S., J. KANE, X.-Y. ZHANG, M. ZHANG and D. J. TIPPER, 1993 Role of the γ component of preprotoxin in expression of the yeast K_1 killer phenotype. Yeast **9:** 251–266.

Communicating editor: M. JOHNSTON