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Spermidine deficiency increases +1 ribosomal frameshifting efficiency and inhibits Ty*1* retrotransposition in *Saccharomyces cerevisiae*

DAVID BALASUNDARAM, JONATHAN D. DINMAN, REED B. WICKNER, CELIA W. TABOR, AND HERBERT TABOR

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

Contributed by Herbert Tabor, September 10, 1993

ABSTRACT **Polyamines have been implicated in nucleic acid-related functions and in protein biosynthesis. RNA sequences that specifically direct ribosomes to shift reading frame in the −1 and +1 directions may be used to probe the mechanisms controlling translational fidelity. We examined the effects of spermidine on translational fidelity by an *in vivo* assay in which changes in β-galactosidase activity are dependent on yeast retrovirus Ty +1 and yeast double-stranded RNA virus L-A −1 ribosomal frameshifting signals. In *spe2*Δ mutants of *Saccharomyces cerevisiae*, which cannot make spermidine as a result of a deletion in the *SPE2* gene, there is a marked elevation in +1 but no change in −1 ribosomal frameshifting. The increase in +1 ribosomal frameshifting efficiency is accompanied by a striking decrease in Ty*1* retrotransposition.**

The polyamines spermidine and spermine have been implicated in a number of physiological functions. Their binding to DNA and RNA suggests that these amines play a significant role in nucleic acid function (reviewed in refs. 1–8). Polyamines have also been implicated in protein synthesis, and studies carried out both *in vivo* and *in vitro* suggest that polyamines can affect translational fidelity (reviewed in refs. 1 and 3–5).

A large number of viruses and transposable elements use ribosomal frameshifting and termination codon suppression for production of fusion proteins (reviewed in refs. 9–12). Two distinct viruses of the yeast *Saccharomyces cerevisiae* use −1 and +1 ribosomal frameshifting to these ends. The L-A double-stranded RNA virus (reviewed in refs. 13 and 14) expresses a 180-kDa gag–pol fusion protein through a −1 ribosomal frameshift mechanism (15) in a manner indistinguishable from that of retroviruses (16). The yeast retrotransposable elements Ty*1* and Ty*3* use a +1 ribosomal frameshift for production of the TYA–TYB fusion protein (17) and gag3–pol3 fusion protein (18), respectively. The ratio of the gag to gag–pol fusion protein, as determined by ribosomal frameshifting efficiency, is critical for propagation of the M_1 satellite double-stranded RNA of the L-A virus, suggesting that ribosomal frameshifting may be a potential target for antiviral compounds (19). Likewise, alterations of the Ty*1* +1 frameshift event reduce its ability to retrotranspose (20, 21).

We have been studying the effects of polyamine depletion on mutants of *S. cerevisiae* that cannot make spermidine and spermine as a result of a deletion in the *SPE2* gene (22, 23), which encodes *S*-adenosylmethionine decarboxylase, a key enzyme in spermidine and spermine biosynthesis (24–26). In this paper, we have investigated the effect of spermidine on ribosomal frameshifting by using an assay system in which changes in β-galactosidase activity are dependent on Ty*1* +1 (17) or L-A −1 ribosomal frameshifting signals (15, 19). Polyamine depletion increases +1 but not −1 ribosomal frameshifting efficiency. In the absence of polyamines, Ty*1* transposition frequency decreases dramatically. Since transposition of Ty*1* requires a programmed +1 ribosomal frameshift for the expression of its gag–pol protein (27), this observation suggests that polyamines are involved in the maintenance of proper reading frame.

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MATERIALS AND METHODS

Strains and Media. *S. cerevisiae* strain Y400 (*MAT*a *ura3-52 trp1-289 leu2 spe2*Δ::*LEU2*) was maintained on H medium without leucine plus 100 μM spermidine and was depleted of amines when required by growing the cells in amine-free medium (22). Unless otherwise indicated, for all experiments, initial spermidine concentrations (at time 0) were 100 μM. Subsequently, 1:10 dilutions were made every 24 hr into fresh amine-free medium. The initial spermidine concentration would thus be diluted 1:10 with each successive dilution.

Plasmids. pJD103 was constructed by inserting the 2.5-kbp *Xho* I fragment of H13 (28) [containing the *HSX1* gene (29) encoding the $tRNA^{Arg(CCU)}$ that reads the AGG codon] into *Xho* I-cleaved pRS426 (30). CCU denotes the anticodon 5′ to 3′ sequence of the loop in this tRNA. pF′8 (Table 1) was modified by use of the Muta-Gene *in vitro* mutagenesis kit (Bio-Rad) and the synthetic oligonucleotide 5′-CGTCGTAC-TCAGCAGCTTAGGCAGAAGTGGTAGGTCTTAC-3′ to create pJD104 containing the Ty*1* +1 ribosomal frameshift signal (underlined). Mutants were confirmed by DNA sequence analysis with modified T7 DNA polymerase (32) (Sequenase v. 2.0; United States Biochemical). In pJD104, *lacZ* is in the +1 reading frame with respect to the initiation codon. The Ty*1* +1 frameshift signal in pJD70 (19) differs from the wild-type Ty*1* +1 ribosomal frameshift signal (17) in that it has a 0 frame AGU (Ser) codon instead of the AGG (Arg) codon (Table 1) and hence is referred to in this paper as Ty*1* derived. All other plasmids (Table 1) were as described.

Transformation of yeast strains with plasmids was by the lithium acetate method (33). Except as otherwise indicated, all *spe2*Δ strains transformed with the plasmid pSPE2-3 (Table 1) are referred to as +Spd and those without this plasmid as −Spd.

Assay for Ribosomal Frameshifting in *spe2*Δ Mutants. Assays for ribosomal frameshifting were carried out essentially as described by Dinman *et al.* (15). In the reporter plasmids (Table 1), synthesis of β-galactosidase required a frameshift, while in the control plasmid pTI25 *lacZ* was in frame with the initiator AUG (see ref. 15). Three individual transformants of each mutant were assayed in duplicate at the indicated times. Activities were expressed as β-galactosidase units [OD_{420}/(OD_{600} per ml of assayed culture × time)].

Percentage ribosomal frameshifting efficiency was calculated by either

Table 1. List of plasmids

Plasmid		Description	Source
pSPE2-3		*SPE2* gene in a *URA3*-selectable multicopy vector that overproduces *S*-adenosylmethionine decarboxylase. *spe2Δ* strains transformed with this plasmid grow with wild-type growth rates.	Ref. 23
pTI25	0 frame control	*lacZ* is in the 0 frame with respect to the initiation codon. No L-A or Ty*1* frameshift signal sequence.	Ref. 15
pTI26	+1 frame control	*lacZ* is in the +1 frame with respect to the initiation codon. No L-A or Ty*1* frameshift signal sequences.	Ref. 15
pF'8	−1 frameshift reporter	*lacZ* is in the −1 frame relative to the initiation codon and L-A sequence. This L-A frameshift signal is G GGU UUA.	Ref. 15
pJD70	+1 frameshift reporter	*lacZ* is in the +1 frame relative to the initiation codon and Ty*1*-derived frameshift signal, CUU AGU C.	Ref. 19
pJD104	+1 frameshift reporter	*lacZ* is in the +1 frame relative to the initiation codon and Ty*1* frameshift signal, CUU AGG C.	This study
pJD103		$tRNA^{Arg(CCU)}$ gene in a *URA3*-based multicopy vector.	This study
pGTy*H3HIS3*	Ty*1* transposition reporter	*HIS3*-marked Ty*1* element fused to the yeast *GAL1* promoter on a *URA3* selectable high-copy plasmid. The Ty*1* element transposes at high frequencies when the promoter is induced by growth on galactose.	Ref. 31

pTI25, pTI26, pF'8, pJD70, and pJD104 were all derived from a common parent plasmid in which transcription is driven from the yeast *PGK1* promoter (15, 19). Translation begins at the first AUG start site and runs through the *Eschericia coli lacZ* gene. The 0 frame is indicated by spaces.

$$\frac{\beta\text{-galactosidase units, } -1 \text{ frameshift} \times 100}{\beta\text{-galactosidase units, 0 frame}},$$

or

$$\frac{\beta\text{-galactosidase units, } +1 \text{ frameshift} \times 100}{\beta\text{-galactosidase units, 0 frame}}.$$

Detection of *lacZ* mRNA. Isolation of total yeast RNA was as described (34). Each sample contained 3.5 μg of total RNA. The *lacZ* (−)-strand RNA probe was made by T7 RNA polymerase runoff transcription of *Xho* I-digested pJD86 (35). Detection of *lacZ*-specific message was as described (34).

Ty*1* Transposition Assay. The assay for Ty*1* transposition from plasmid to host chromosome was as described by Garfinkel *et al.* (31). The strain Y409-3 (*MATa ura3-52 trp1-289 his3 leu2 spe2Δ::LEU2*) was transformed with the *URA3*-containing plasmid, pGTy*H3HIS3* (Table 1). A stable URA^+ transformant (Y417) was grown with and without spermidine for 2 days to partially deplete the culture of amines in the latter case. The cells were washed in H medium without spermidine, resuspended in H medium with galactose and without leucine and uracil, and then inoculated into H medium with galactose and without leucine and uracil at an OD_{600} of 0.2 and incubated at 22°C for 5 days in the presence of spermidine concentrations ranging from 100 μM to 1 pM. Control cells were not previously depleted and were supplemented with 100 μM spermidine. Aliquots from these cultures were then streaked on plates of H medium without uracil and leucine, containing spermidine (100 μM) and incubated at 30°C for 5 days. Cells from these plates were streaked for single colonies onto YPAD (yeast/peptone/dextrose/adenine sulfate) to allow loss of pGTy*H3HIS3*. Ura^- colonies were identified by replica plating from YPAD onto plates with H medium without uracil. Transposition events were detected by the ability of the Ura^- colonies to grow on plates with H medium without histidine. Transposition efficiency is defined as the number of His^+/Ura^- colonies divided by the total number of Ura^- colonies.

RESULTS

There Is No Significant Change in −1 Ribosomal Frameshifting Efficiency in Amine-Depleted *spe2Δ* Cells. Y400 (*spe2Δ*) cells transformed with pF'8 (−1 ribosomal frameshift reporter; Table 1) were grown in the absence of amines, and frameshifting efficiency was monitored during amine depletion. The −1 ribosomal frameshifting efficiencies remained fairly constant over a 118-hr period (Table 2). Control cells containing pF'8 and pSPE2-3 (Table 1) also showed no change in −1 frameshift efficiency (Table 2). These results indicate that spermidine depletion had no effect on −1 ribosomal frameshifting. Although the observed efficiencies of −1 ribosomal frameshifting were higher than those reported (15, 19), strain-specific variations have been noted (unpublished observations) and further genetic analysis is required to determine the cause of such variations.

Elevated +1 Frameshifting During Amine Depletion of *spe2Δ* Cells. To examine the effects of amine depletion on +1 ribosomal frameshifting, a plasmid (pJD104; Table 1) in which the Ty*1* +1 ribosomal frameshifting signal is required for *lacZ* expression was used. The *spe2Δ* strain, Y400 containing pJD104, or the 0 frame control plasmid, pTI25, was grown in the absence and presence of pSPE2-3, and β-galactosidase activity was assayed at the indicated time points (Fig. 1). There was a steady increase in +1 ribosomal frameshifting efficiency with time in the absence of amines (Fig. 1*A*, curve b). The control strain containing pSPE2-3 did not exhibit any change in ribosomal frameshifting efficiency (Fig. 1*A*, curve a). The elevation of +1 frameshifting effi-

Table 2. No significant change in −1 ribosomal frameshifting efficiency in amine-deficient *spe2Δ* cells

Duration, hr	−1 ribosomal frameshifting, % efficiency	
	− Spd	+ Spd
20	11.7	11.7
44	11.8	8.3
68	15.3	8.3
90	12.6	9.6
118	10.7	9.5

Strain Y400 (*spe2Δ*) without (− Spd) and with (+ Spd) the plasmid pSPE2-3 (Table 1) was transformed with 0 frame control and −1 frameshift reporter plasmids (pTI25 and pF'8, respectively; Table 1). β-Galactosidase activity was measured at the indicated times and % efficiency of −1 ribosomal frameshifting was calculated as described.

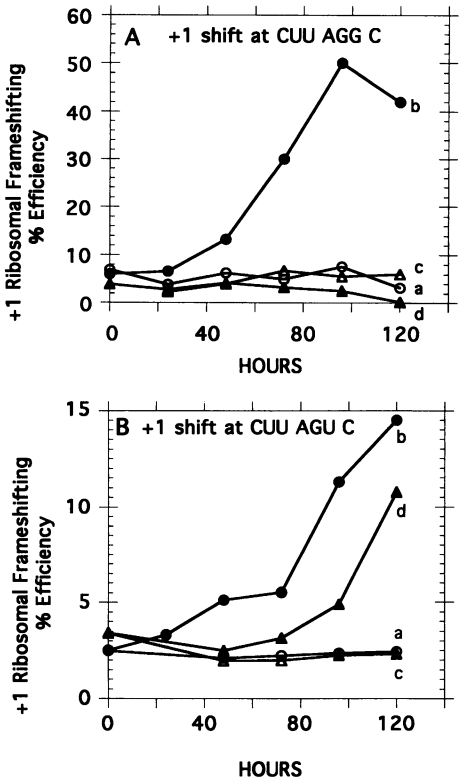

FIG. 1. Increase in +1 ribosomal frameshifting efficiency during polyamine depletion of *spe2*Δ mutants and its inhibition by a multicopy plasmid carrying the $tRNA^{Arg}$ gene recognizing AGG. Strains of Y400 (*spe2*Δ) harboring the +1 frameshift reporter plasmids pJD104 (*A*) or pJD70 (*B*) were grown in purified medium with and without a multicopy plasmid, pSPE2-3, or addition of spermidine (100 μM). The above transformants were also transformed with the multicopy plasmid pJD103 carrying the $tRNA^{Arg}$ gene recognizing AGG (Table 1). β-Galactosidase activity was measured at the indicated times and % efficiency of +1 ribosomal frameshifting was determined as described. (*A*) ○, pJD104 + pSPE2-3; ●, pJD104 (no amines); △, pJD104 + pJD103 + 100 μM spermidine; ▲, pJD104 + pJD103 (no amines). (*B*) ○, pJD70 + pSPE2-3; ●, pJD70 (no amines); △, pJD70 + pJD103 + 100 μM spermidine; ▲, pJD70 + pJD103 (no amines).

ciency began in the early stages of amine depletion and increased progressively.

Plasmid pJD70 (Table 1) contains a Ty*1*-derived +1 frameshift signal having a 0 frame AGU (Ser) codon at the A-site instead of the AGG (Arg) codon. Strain Y400 carrying pJD70 was grown with and without the pSPE2-3 plasmid. Similar to the results seen with pJD104, polyamine depletion resulted in increased +1 ribosomal frameshifting with this Ty*1*-derived slippery site (Fig. 1*B*, curve b).

To eliminate the possibility that such an increase in +1 frameshifting efficiency may be related to the nonspecific instability of the ribosome in the absence of saturating amounts of spermidine, we examined frameshifting efficiencies in the absence of the +1 frameshift signal. In pTI26 (Table 1), the *lacZ* gene is in the +1 reading frame, relative to the translational start site, but without the intervening, specific frameshift signal. Y400 containing pTI26 with and without pSPE2-3 was grown in amine-free medium and assayed for β-galactosidase activity. No changes in +1 frameshifting efficiencies were noted, which were all <0.4% (results not shown), indicating that the observed increases in +1 ribosomal frameshifting in Fig. 1 were due to specific frameshift signals.

The increase in +1 ribosomal frameshifting efficiencies in −Spd cells was not related to the effects of polyamine deficiency on cell growth or protein synthesis through the first 96 hr. The growth of +Spd and −Spd cells was essentially equal until 96 hr of amine depletion (Fig. 2*A*). The specific activities of β-galactosidase from pTI25 in these cells also remained relatively constant during this time (Fig. 2*B*).

The Effect of Polyamine Depletion Occurs at the Posttranscriptional Level. Total RNA from cells containing pJD104, with and without pSPE2-3, was extracted at the time points indicated in Fig. 2 and equal amounts of RNA were analyzed on denaturing gels, transferred to nitrocellulose, and hybridized with a *lacZ* (−)-strand-specific probe. No significant differences were observed in steady-state levels of *lacZ* RNA between −Spd and +Spd cells, indicating that *lacZ*-specific transcriptional rates were essentially equivalent in −Spd and +Spd cells (results not shown).

Overproduction of $tRNA^{Arg(CCU)}$ Suppresses +1 Frameshifting During Amine Depletion. The +1 ribosomal frameshifting in Ty*1* occurs during a translational pause at the 0 frame AGG (Arg) codon of the frameshift signal (17). This is induced by the low availability of the $tRNA^{Arg(CCU)}$ gene, which is encoded by a single copy gene (28) denoted *HSX1* (29). Introduction of multiple copies of this gene strongly inhibited +1 ribosomal frameshifting at the Ty*1* +1 ribosomal frameshifting signal (17).

The *spe2*Δ strain Y400 carrying the Ty*1* +1 frameshift reporter plasmid (pJD104) or the control plasmid (pTI25) was transformed with pJD103, a high copy vector carrying the $tRNA^{Arg(CCU)}$ gene (Table 1) and grown with and without added spermidine. No increase in +1 ribosomal frameshifting was observed with pJD103 either +Spd (Fig. 1*A*, curve c) or −Spd (Fig. 1*A*, curve d). These observations confirm that the elevated +1 ribosomal frameshifting observed in spermidine-depleted *spe2*Δ cells occurs by the same mechanism reported

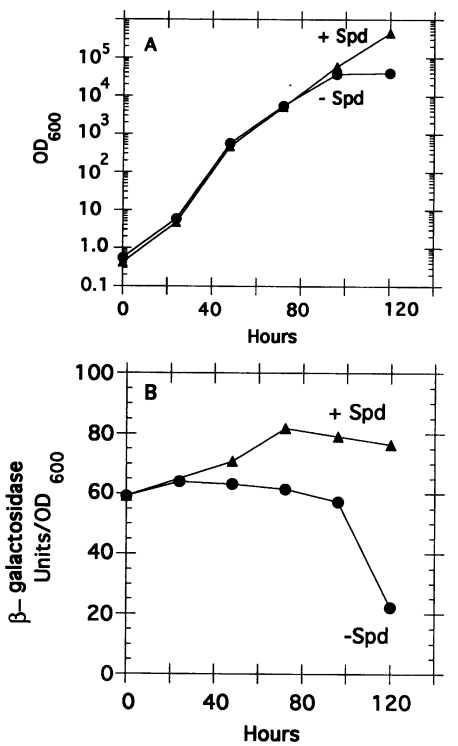

FIG. 2. Growth and β-galactosidase activity of *spe2*Δ mutants during polyamine depletion. The *spe2*Δ strain Y400 containing the 0 frame control plasmid (pTI25; Table 1) without (− Spd) and with (+ Spd) a plasmid overproducing the *SPE2* gene (pSPE2-3; see Table 1) was grown in amine-free medium. At the indicated times, the optical densities (OD_{600}) corrected for the appropriate dilution (*A*) and β-galactosidase activities from pTI25 (*B*) were determined, as described.

for normal cells (17). We also examined whether $tRNA^{Arg(CCU)}$ suppresses +1 ribosomal frameshifting in a construct [0 frame AGU (Ser) codon; pJD70] that differs from the wild-type Ty*1* +1 ribosomal frameshift signal [0 frame AGG (Arg) codon; pJD104]. Y400 carrying either the Ty*1*-derived +1 frameshift reporter (pJD70) or the control plasmid (pTI25) was transformed with pJD103 (Table 1) and grown with and without added spermidine. pJD103 reduced the increase in +1 ribosomal frameshifting efficiency (Fig. 1*B*, curve d) but not as dramatically as with pJD104 (Fig. 1*A*, curve d).

Effect of Spermidine on the Frequency of Ty*1* Transposition. The ratio of gag to gag–pol as determined by −1 ribosomal frameshifting efficiency is critical for the propagation of M_1, a satellite virus of L-A (19, 35). Expression of an analogous gag–pol fusion protein of the retrotransposon yeast Ty*1* takes place through a +1 ribosomal frameshift (27) and alteration of +1 frameshift efficiency by altering the $tRNA^{Arg(CCU)}$ concentration diminishes Ty*1* transposition (20, 21). Since spermidine specifically increases +1 ribosomal frameshifting efficiency, we studied the effect of amine depletion on the frequency of Ty*1* transposition as an assay for viral propagation. Ty*1* was induced to transpose in a *spe2*Δ mutant (Y417; see *Materials and Methods*) in the presence of various amounts of spermidine by using a plasmid (pGTy*H3HIS3*; Table 1) in which a *HIS3*-marked Ty*1* is transcribed from a *GAL1* promoter (31). Since Ty*1* retrotransposition requires that the galactose-induced transcript pass through a viral intermediate, all cells in which a transposition occurred (i.e., Ura^-, His^+) must have been able to propagate the viral form of Ty*1*. After 5 days in galactose, control cells grew to an OD_{600} of 1.44 (≈2.5 doublings from an initial OD_{600} of 0.2), whereas the cells that were previously amine depleted and placed in 100 μM to 1 pM spermidine uniformly grew to an OD_{600} of 0.4–0.5 (≈1.0 doubling). We observed a marked decrease in Ty*1* transposition frequency, from ≈41% in control cells (continuously supplemented with 100 μM spermidine) to ≈1% at an initial concentration of 1 pM spermidine, a total decrease of 40-fold (Fig. 3).

DISCUSSION

The results presented in this paper support the involvement of spermidine in +1 ribosomal frameshifting. The +1 ribosomal frameshifting efficiencies using Ty*1* and Ty*1*-derived frameshift signals increased progressively during growth in amine-free medium, while no such effect was observed on −1 ribosomal frameshifting. The elevation in +1 ribosomal frameshifting efficiency was not due to the effects of polyamine deficiency on the ability of cells to grow since doubling times in the absence of spermidine began to fall only after ≈96 hr. That no increase in +1 ribosomal frameshifting efficiency was seen with the control plasmid pTI26 indicated that the effect of the *spe2* mutation was specifically in response to the Ty*1* and Ty*1*-derived +1 ribosomal frameshift signals.

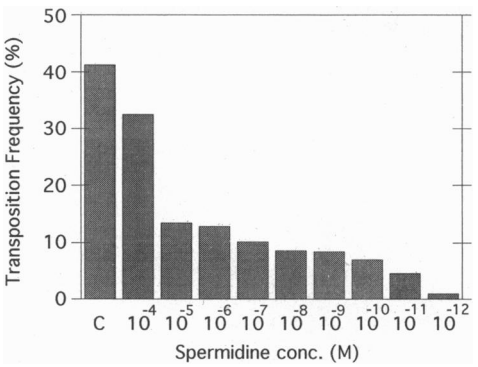

FIG. 3. Effect of spermidine deficiency on Ty*1* retrotransposition. Y417 was grown with and without spermidine for 2 days to partially deplete the culture of amines, after which cells from both cultures were suspended in H/galactose medium (initial OD_{600} of 0.2) at the indicated spermidine concentrations. The assay for Ty*1* transposition was as described by Garfinkel *et al.* (31). Between 70 and 118 Ura^- colonies were assayed for His^+ reversion. Transposition frequency is the percentage of cells that have had one or more transposition events. C, control cells that were supplemented with 100 μM spermidine and not previously depleted of polyamines.

The +1 and −1 ribosomal frameshifting are evoked by different mechanisms and occur at different stages in the elongation pathway (for review, see ref. 12). Although both require a translational pause, in −1 frameshifting the pause is induced by an RNA pseudoknot (15, 36–39), whereas a +1 ribosomal frameshift event is generated as a result of a translational pause brought about by the low availability of a rare tRNA (refs. 17 and 18; for other references, see ref. 40). The +1 ribosomal frameshifting has been proposed to occur when a peptidyl-tRNA occupies the ribosomal P-site and the A-site is unoccupied (17)—i.e., at the time of aminoacyl-tRNA selection. The −1 ribosomal frameshifting occurs later in the elongation process, when both the P- and A-sites are occupied by tRNAs (16). As previously shown (17), we have also found that overproduction of the "rare" $tRNA^{Arg(CCU)}$ severely inhibited frameshifting. That the *spe2* mutation specifically affects +1 rather than −1 ribosomal frameshifting efficiencies may serve to define the site of action of polyamines in the elongation process. Spermidine may be involved in the selection and/or insertion of cognate tRNA at the ribosomal A-site. Alternatively, spermidine may be required for the synthesis and/or aminoacylation of this rare tRNA. Indeed, a number of studies, such as those found in refs. 41–44, have been reported on the effect of spermidine on tRNA and especially on its effect on aminoacylation of tRNA *in vitro*. Either way, limiting concentrations of spermidine would increase the length of the ribosomal pause, thereby increasing ribosomal frameshifting efficiencies.

The Ty*1*-derived +1 frameshift site in pJD70 retains the P-site CUU to UUA slippery sequence but the second codon of the slippery site has a 0 frame AGU (Ser) instead of the AGG (Arg). Like the AGG (Arg) codon, the AGU (Ser) is a low-abundance codon (45). The cognate tRNA is also a minor species (46) and provides the basis for the required translational pause in +1 ribosomal frameshifting by Ty*3* (18). That there was no effect of overproduction of $tRNA^{Arg(CCU)}$ before any polyamine depletion indicates that cells containing the appropriate polyamine concentrations utilize the cognate tRNA at the correct codon, but cells with low to no polyamine levels would prefer to insert a near cognate tRNA when the latter is presented in abundance rather than shift reading frame. The observation that spermidine inhibits +1 frameshifting irrespective of the absence or presence of a likely suppressor, here $tRNA^{Arg(CCU)}$, may be related to the previous observation that polyamines are required in *Escherichia coli* for the efficient translation of an amber codon even in a strain carrying a suppressor gene (47).

The suppression of +1 ribosomal frameshifting by overproduction of $tRNA^{Arg(CCU)}$ resulted in decreased Ty*1* transposition efficiency (20). On the other hand, cells lacking $tRNA^{Arg(CCU)}$ exhibited a 3- to 17-fold increase in +1 frameshifting efficiency and at least a 50-fold decrease in Ty*1* transposition (21). Dinman and Wickner (19) hypothesized that the ratio of gag to gag–pol as determined by ribosomal frameshifting efficiency is critical for viral particle assembly. In the Ty*1* transposition assay, the transcript produced from the plasmid must be translated and go through a viral intermediate before transposition can be effected (48). It is important to note that all the partially depleted cells went through more or less the same number of generations when

incubated in H/galactose medium containing 100 μM to 1 pM spermidine. Thus, the decrease in transposition frequency is not due to the cell's inability to grow but rather suggests that increasing the efficiency of +1 ribosomal frameshifting changes the ratio of TYA to TYA–TYB proteins, consequently interfering with viral particle assembly. The effect of polyamines on the maintenance of reading frame may thus have implications for the *in vivo* propagation of genetic elements in which the efficiencies of ribosomal frameshifting are critical.

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