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SPE1 and *SPE2*: Two Essential Genes in the Biosynthesis of Polyamines That Modulate +1 Ribosomal Frameshifting in *Saccharomyces cerevisiae*

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We previously showed that a mutant of *Saccharomyces cerevisiae*, which cannot make spermidine as a result of a deletion in the *SPE2* gene (*spe2Δ*), exhibits a marked elevation in +1 ribosomal frameshifting efficiency in response to the Ty1 frameshift sequence, CUU AGG C. In the present study, we found that spermidine deprivation alone does not result in increased +1 ribosomal frameshifting efficiency. The high level of +1 ribosomal frameshifting efficiency in *spe2Δ* cells is the result of the combined effects of both spermidine deprivation and the large increase in the level of intracellular putrescine resulting from the derepression of the gene for ornithine decarboxylase (*SPE1*) in spermidine-deficient strains.

Certain genes have evolved mechanisms by which a single protein is synthesized from two different reading frames on an mRNA template. The synthesis of such proteins is regulated by the amount of ribosomal frameshifting, which permits these different frames to be read as a single protein. Such mechanisms involve (i) mRNA containing recoding, or frameshifting signals and secondary or tertiary modifications; (ii) the ribosome and its ability to hop, frameshift, or read through termination codons; and finally (iii) rare cognate aminoacyl-tRNAs, whose scarcity dictates the stalling of the ribosome in response to the specific frameshift signals (for reviews, see references 1, 9, 12, 13, and 16).

We have been examining ribosomal frameshifting in spermidine- and spermine-deficient mutants of *Saccharomyces cerevisiae*. Using the Ty1 frameshift sequence, CUU AGG C (4), as well as a Ty3-like frameshift sequence, CUU AGU C (10), in a reporter system, we previously showed that depletion of spermidine and spermine in mutants with a deletion in the *SPE2* gene (*spe2Δ*) causes the translating ribosome to shift the reading frame in the +1 direction at very high levels (2).

We had postulated that spermidine might be involved in the maintenance of proper reading frames through its effect either on the selection and/or insertion of cognate tRNAs at the ribosomal A site or on the synthesis and/or aminoacylation of rare tRNAs (2). However, since *spe2*$^-$ mutants of *S. cerevisiae* become markedly derepressed for putrescine biosynthesis during the development of spermidine and/or spermine deprivation (3, 5, 6, 21), we decided to investigate the effect of elevated putrescine levels on the efficiency of +1 ribosomal frameshifting.

Yeast strains, media, and growth conditions. *S. cerevisiae* Y450 (*MATa arg4 ura3-52 trp1-289 thr1 leu2 spe1Δ::LEU2 spe2Δ::LEU2*) and Y400 (*MATa ura3-52 trp1-289 leu2 spe2Δ::LEU2*) were maintained on H medium minus leucine plus 10^{-4} M spermidine and were depleted of amines when required by growing the cells in amine-free medium at 30°C as described below. Strains transformed with plasmids were grown in H medium minus leucine and tryptophan or minus leucine, tryptophan, and uracil.

The elevated +1 ribosomal frameshifting efficiency exhibited by the *SPE1 spe2Δ* mutant is not found in a *spe1Δ spe2Δ* double mutant during polyamine depletion. *SPE1 spe2Δ* and *spe1Δ spe2Δ* cells, transformed with pJD104 and pT125 (+1 ribosomal frameshift reporter and 0-frame control plasmids, respectively) (Table 1), were grown in 10^{-4} M spermidine (zero time) and then successively (every 24 h) diluted 1:10 into amine-free medium. Under these conditions, the growth of all of the cultures (as determined by optical density measurements at 600 nm) did not decrease until after 4 days, when the calculated spermidine concentration was 10^{-8} M; i.e., no difference in the growth curves was seen throughout the experiment for the *SPE1 spe2Δ* and *spe1Δ spe2Δ* cells.

Assays for +1 ribosomal frameshifting were carried out by measuring β-galactosidase in cells containing the reporter plasmids (Table 1) as previously described (2). In strains carrying the reporter plasmid pJD104, synthesis of β-galactosidase requires a +1 ribosomal frameshift relative to the initiator AUG, while in the control plasmid pT125, *lacZ* is in frame with the initiator AUG. *SPE1 spe2Δ* cells containing pJD104 showed a marked increase in +1 ribosomal frameshifting efficiency during the course of spermidine depletion (Fig. 1), as we had reported previously (2). However, the *spe1Δ spe2Δ* cells containing the same reporter plasmid showed little or no increase in +1 ribosomal frameshifting efficiency during the course of spermidine depletion (Fig. 1). Control experiments with the same strains (i.e., *spe1Δ spe2Δ* and *SPE1 spe2Δ* cells) containing the 0-frame control plasmid pTI25 showed no differences in the amounts of β-galactosidase synthesized.

Since in the absence of a functional *SPE1* gene there is no measurable increase in +1 ribosomal frameshifting efficiency despite the decrease in spermidine concentration, these results indicated that the presence of an active *SPE1* gene (and the resultant increase in putrescine levels) is required for the increased +1 ribosomal frameshifting efficiency observed in the absence of spermidine. We measured the putrescine concentration of the spermidine-depleted *SPE1 spe2Δ* cells, and as expected from our previous studies, we found that the intracellular putrescine levels increased during the develop-

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TABLE 1. Plasmids used in this study

Plasmid	Description	Source or reference
pSPE1c	A 2,955-bp *Kpn*I-*Eco*RI fragment containing the *SPE1* gene (23) derived from the original pSPE-12 plasmid supplied by Fonzi and Sypherd (11) inserted into the *URA3* selectable multicopy vector pVT101U of Vernet et al. (22). In this construction, transcription is driven by the yeast *ADH1* promoter, which results in overexpression of ornithine decarboxylase constitutively.	This study
pTI25 (0-frame control)	*lacZ* is in the 0 frame with respect to the initiation codon; no Ty1 frameshift signal sequence is present.	(2)
pJD104 (+1 ribosomal frameshift reporter)	*lacZ* is in the +1 frame relative to the initiation codon and the Ty1 frameshift signal, CUU AGG C.	(2)

ment of the spermidine deficiency, presumably as a result of derepression of the *SPE1* gene (Table 2).

Introduction of the *SPE1* gene on a high-copy-number plasmid into the *spe1*Δ *spe2*Δ double mutant results in elevated +1 ribosomal frameshifting efficiency. To confirm the importance of intracellular putrescine for +1 ribosomal frameshifting, a *spe1*Δ *spe2*Δ strain with and without a plasmid containing a constitutively expressed *SPE1* gene (pSPE1c; Table 1) was assayed for +1 ribosomal frameshifting. The strain containing the pSPE1c plasmid had a high level of intracellular putrescine (Table 2). As shown in Fig. 1, for this strain there was a steady increase in +1 ribosomal frameshifting efficiency with decreasing spermidine concentrations, whereas there was no effect on the isogenic control without the overproducing plasmid. Notably, even at zero time, the +1 ribosomal frameshifting efficiency was two- to threefold higher than that observed for either *spe1*Δ *spe2*Δ or *SPE1 spe2*Δ cells (Table 2). The higher +1 ribosomal frameshifting efficiencies due to overexpression of ornithine decarboxylase were not related to the effects of the plasmid pSPE1c upon growth or protein synthesis per se, since growth and specific activities of β-galactosidase for the cells were the same in the presence and absence of the overexpression plasmid.

We have been unable to duplicate the effect of the pSPE1c plasmid by the addition of putrescine to the medium; i.e., no increase in +1 ribosomal frameshifting efficiency was observed when the *spe1*Δ *spe2*Δ mutant was grown in the presence of 10^{-2} or 10^{-4} M putrescine. It is possible that the inability of exogenous putrescine to promote +1 ribosomal frameshifting is due to problems related to the uptake or the compartmentalization of exogenous putrescine.

The marked increase in ribosomal frameshifting efficiency requires both a rise in putrescine levels and a decrease in spermidine levels. Amine analysis of the strain containing the constitutively expressed ornithine decarboxylase gene (in pSPE1c), grown in 10^{-4} M spermidine (i.e., at zero time), showed a very high internal concentration of putrescine and a moderately high concentration of spermidine (Table 2). After spermidine depletion for 5 days, however, analysis showed high levels of putrescine but no detectable internal spermidine (Table 2).

If putrescine alone were involved in the modulation of +1 ribosomal frameshifting efficiency, one would have expected to see an extremely high level of frameshifting at zero time for this strain containing the constitutively expressed *SPE1* gene, since the internal putrescine concentration was very high. However, the level of frameshifting efficiency was low compared with the extent of +1 ribosomal frameshifting observed after spermidine depletion. Our observations therefore suggest that the relative concentrations of spermidine and putrescine may play a crucial role in determining the efficiency of +1 ribosomal frameshifting.

One might postulate that during amine depletion the high putrescine level competes with the low residual concentration of spermidine, and, in response to the specific frameshifting signal, raises the +1 ribosomal frameshifting efficiency to high levels. A possible reason for this effect of putrescine is that under these conditions (i.e., low spermidine concentration), a high putrescine concentration affects the structure of the ribosome or of the aminoacyl-tRNA-mRNA complex. Indeed, earlier work suggested that putrescine may affect the structural integrity and function of ribosomes (14, 15, 19, 20).

Alterations in either −1 or +1 ribosomal frameshifting efficiencies are known to be deleterious to viral propagation in

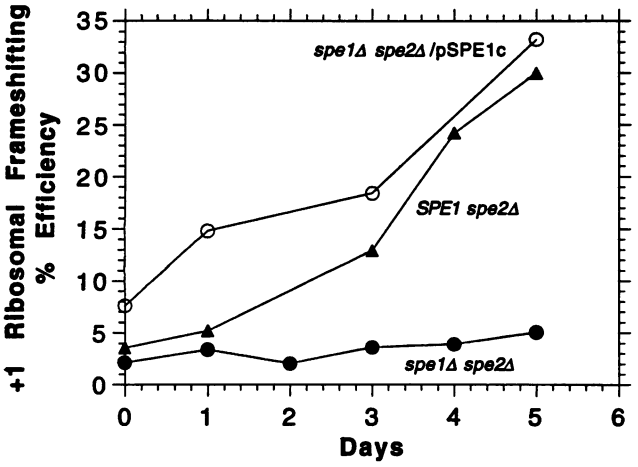

FIG. 1. Elevated efficiency of +1 ribosomal frameshifting in *spe2*Δ mutants during spermidine depletion requires expression of the *SPE1* gene. Isogenic *spe1*Δ *spe2*Δ, *SPE1 spe2*Δ, and *spe1*Δ *spe2*Δ/pSPE1c strains were transformed with the 0-frame control and +1 ribosomal frameshift reporter plasmids (pTI25 and pJD104, respectively; Table 1). Initial spermidine concentrations (at zero time) were 10^{-4} M. Subsequently, 10-fold dilutions were made every day into fresh amine-free medium. The calculated external spermidine concentration at the end of 5 days was 10^{-9} M. Aliquots were withdrawn at the indicated intervals and tested for +1 ribosomal frameshifting by β-galactosidase assays as previously described (2). Four independent transformants of each mutant were assayed in duplicate at the indicated times. Activities are expressed as β-galactosidase units (optical density at 420 nm per hour per milliliter of culture/optical density at 600 nm of the culture). Percent ribosomal frameshifting efficiency was calculated as follows: (β-galactosidase units [+1 frameshift]/β-galactosidase units [0 frame]) × 100.

TABLE 2. Effect of the constitutively expressed *SPE1* gene on internal putrescine and spermidine levels and on +1 ribosomal frameshifting efficiency in a *spe1*Δ *spe2*Δ double mutant[a]

S. cerevisiae mutation(s)	Amine concn[b]				+1 Ribosomal frameshifting (% efficiency)	
	Zero time[c]		After amine depletion[d]		Zero time	After amine depletion
	Putrescine	Spermidine	Putrescine	Spermidine		
*SPE1 spe2*Δ	ND	2.7	0.84	ND	3.6	30
*spe1*Δ *spe2*Δ	ND	3.7	ND	ND	2.1	5.1
*spe1*Δ *spe2*Δ/pSPE1c	60	8.4	1.05	ND	7.6	33

[a] *SPE1 spe2*Δ, *spe1*Δ *spe2*Δ, and *spe1*Δ *spe2*Δ/pSPE1c cells containing either a 0-frame control or a +1 ribosomal frameshift reporter plasmid (Table 1) were grown in 10^{-4} M spermidine (Fig. 1). These cells were diluted into fresh amine-free medium each day so as to obtain a final spermidine concentration (calculated) of 10^{-9} M after 5 days. (Fig. 1). The cells were harvested and assayed for amines by high-pressure liquid chromatography (HPLC) (3) and for +1 ribosomal frameshifting efficiency (2).

[b] Micromoles per gram (wet weight) of cells. ND, not detectable by HPLC. The limit of detection with our methods is 0.02 μmol/g (wet weight) of cells.

[c] Corresponds to 0 days in Fig. 1. The external spermidine concentration was 10^{-4} M.

[d] Corresponds to 5 days in Fig. 1. The calculated spermidine concentration (in the culture) was 10^{-9} M.

S. cerevisiae (2, 7, 17, 24). Indeed, the isolation of maintenance-of-frame mutants on the basis of their ability to increase the efficiency of −1 ribosomal frameshifting (8) underscores the importance of chromosomal genes affecting this process. Our results imply that in an analogous manner, the two chromosomal genes, *SPE1* and *SPE2*, could modulate the efficiency of +1 ribosomal frameshifting. It is of relevance that expression of the chromosomal gene encoding antizyme, a protein implicated in the regulation of mammalian ornithine decarboxylase, requires a +1 ribosomal frameshift which is in turn regulated by the concentration of polyamines, as found by Hayashi and colleagues (cited in reference 13) and by Rom and Kahana (18).

The importance of the putrescine/spermidine ratio in modulating ribosomal frameshifting should be considered in developing antiviral strategies, especially with those viruses for which the efficiencies of +1 ribosomal frameshifting might be critical for in vivo propagation.

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