Effect of seminal plasmin on rRNA synthesis in *Saccharomyces cerevisiae*

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Seminal plasmin, the highly basic, antimicrobial protein, isolated from bull semen, was found to inhibit the transcription of ribosomal RNA in yeast. Protein synthesis and processing of rRNA remained unaffected. Seminal plasmin appears to be useful for studies of the biosynthesis of yeast rRNA in pulse-chase experiments.

Yeast rRNA synthesis *Seminal plasmin* *Inhibition*

1. INTRODUCTION

Seminal plasmin (SPL) is a highly basic protein with a molecular mass of 6385 Da [1] isolated from bull semen [2–4]. The amino acid sequence was recently established [4]. In vivo and in vitro studies have shown that SPL inhibits the growth and the synthesis of RNA in *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida albicans* [2–4]. In yeast approx. 10-times higher concentrations of SPL are required to inhibit completely the growth and RNA synthesis when compared to *E. coli*.

We have isolated fragile mutants of *S. cerevisiae* which grow only in the presence of osmotic stabilisers [5]. When suspended in hypotonic solutions the cells lyse immediately. The fragile cells show sensitivity to different inhibitors of RNA synthesis, known to have little effect on wild-type yeast [5,6].

We present here evidence for an exceptionally high sensitivity of the fragile yeast mutants towards SPL which might be used to study the biosynthesis of RNA in yeast cells.

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2. MATERIALS AND METHODS

Strains and media: the following *S. cerevisiae* strains were used: S288C, A346 and 1278 are parental strains of the fragile mutants VY1160, SY15 and VY481, respectively. The origin, selection procedure and genotype of the strains have been described [5]. Cells were grown in YGS medium (1% yeast extract, 3% glucose, 10% sorbitol) at 30°C till middle-exponential phase and then used in experiments. Determination of the UTP pool [7], and isolation and analysis of rRNA in denaturing agar–urea gels followed [8]. SPL was isolated as in [4].

3. RESULTS AND DISCUSSION

Table 1 shows the incorporation of radioactive precursors into RNA of cells treated with SPL. The wild-type strains A346, S288C and 1278 are sensitive to high concentrations of SPL. In contrast, in the fragile mutants SY15, VY1160 and VY481, concentrations of SPL as low as 5 μg/ml inhibited the incorporation of [^{3}H]uracil into RNA by about 50%. Usually 50-times more SPL is needed to achieve the same effect in the corresponding parental strains. The sensitivity to SPL parallels

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Table 1

Sensitivity of yeast strains to seminal plasmin

Strain	Lysis[a] (%)	SPL[b] (μg/ml)					
		0	5	10	20	60	200
A346	1	89500	91000	89300	88600	89100	46500
		100%	102%	100%	99%	100%	52%
SY15	45	35800	20200	8200	3220	460	240
		100%	56%	23%	9%	1.3%	0.7%
SY15	2	53200	51800	54100	48500	47800	25200
revertant		100%	97%	102%	91%	90%	47%
S288C	1	26900	25800	27300	26500	27100	15600
		100%	96%	102%	99%	101%	58%
VY1160	65	13200	4900	2370	800	70	80
		100%	31%	18%	6%	0.5%	0.5%
VY1160	1	22600	22800	20100	19600	18200	10700
revertant		100%	100%	89%	88%	81%	47%
1278	1	385000	380000	382000	363000	385000	161700
		100%	99%	99%	94%	93%	42%
VY481	85	60000	7200	2400	480	180	200
		100%	12%	4%	0.8%	0.3%	0.3%
VY481		98500	102700	96300	87900	86100	36300
revertant	1	100%	104%	98%	89%	87%	37%

[a] Percent lysed cells in water determined as in [6]

[b] Incorporation of [^{3}H]uracil into trichloroacetic acid-insoluble material after labelling of 0.5 ml culture with 10 μCi/ml [^{3}H]uracil in the presence of SPL for 30 min

well the fragility of the cells, being the highest in VY481. The revertants were found to lose sensitivity towards SPL (table 1), suggesting a direct connection between cell fragility and increased sensitivity to SPL. The fragile mutants belong to different complementation groups [5] and have different lesions in the cell wall structure [9,10]. Therefore, the increase in the sensitivity to SPL appears to result from increased permeation and indicates that the growth inhibition of yeast cells is not based on receptor-mediated uptake.

Fig.1 shows the kinetics of incorporation of labelled precursors into ribosomal RNA (rRNA) and proteins in VY481 cells. At 5 μg/ml SPL synthesis of rRNA occurred during the first 20 min at a decreased rate. At 10 μg/ml SPL led to an immediate and total inhibition of rRNA synthesis. As shown in fig.1, SPL had no effect on protein synthesis. Similar results were obtained with the two other fragile mutants VY1160 and SY15 (not shown); a complete arrest of rRNA synthesis could be achieved at 15 μg/ml SPL. We show below that

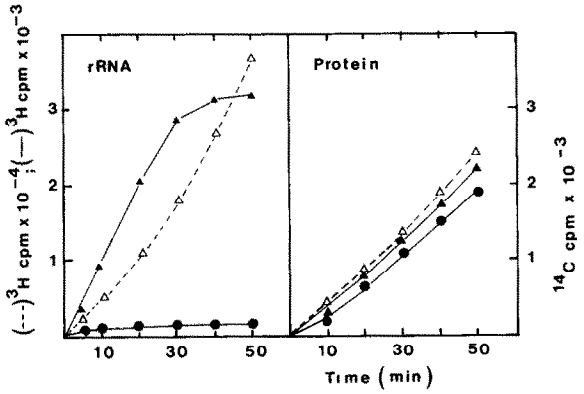

Fig.1. Synthesis of rRNA and protein in VY481 cells in the presence of SPL. Aliquots of a culture of VY481 cells were labelled with 10 μCi/ml [^{3}H]uracil or 2 μCi/ml ^{14}C-labelled amino acids in the presence of 5 μg/ml SPL (▲), or 10 μg/ml SPL (●). A control contained no SPL (△). At timed intervals, rRNA was purified and its radioactivity expressed as cpm/50 μg rRNA. Protein synthesis was measured as ^{14}C-labelled amino acid incorporated into hot trichloroacetic acid-insoluble material and is given as cpm/0.5 ml.

the fragile mutants can be employed to study the effect of SPL on transcription and processing of rRNA in yeast without concomitant inhibition of protein synthesis.

The inhibition of rRNA synthesis in yeast effects the nucleoside triphosphate pools [7,8]. In the presence of 5 μg/ml SPL, the uptake of [^{3}H]uracil into the UTP pool of VY481 cells was 83%, and at 10 μg/ml SPL, 58% relative to the control. The values obtained were used to correct the data observed for the synthesis of rRNA in presence of SPL.

We quantitatively analysed rRNA transcription by pulse-labelling VY481 cells with [^{3}H]uracil in the absence and presence of SPL (fig.2). In all rRNA samples [^{3}H]uracil is present exclusively in the precursors of rRNA molecules (pre-rRNA). No mature 25 S and 18 S rRNA is synthesised after such a brief pulse which permits accurate estimation of the synthesis of the 37 S primary transcript; the dpm ([^{3}H]uracil) in the 37 S peak were normalised to dpm ([^{14}C]uracil) in the stable 25 S rRNA from a long-duration labelling experiment. Treatment with 5 μg/ml SPL caused an inhibition of the synthesis of 37 S pre-rRNA by 84%, and at 10 μg/ml SPL, this synthesis ceased completely. These results correspond well with the inhibition of [^{3}H]uracil incorporation (table 1) and with previous in vitro studies (unpublished) where an inhibition of RNA polymerase I from *S. cerevisiae* by SPL was established.

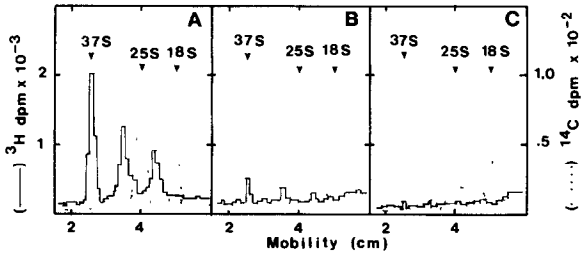

Fig.2. Analysis of rRNA. VY481 cells were grown overnight in the presence of 0.01 μCi/ml [^{14}C]uracil and the culture divided into 3 parts. Part A was labelled with 20 μCi/ml [^{3}H]uracil for 90 s; parts B and C were treated for 10 min with 5 or 10 μg/ml SPL, respectively, and then labelled with 20 μCi/ml [^{3}H]uracil for 90 s. rRNA was isolated and analysed in denaturing agar–urea gels.

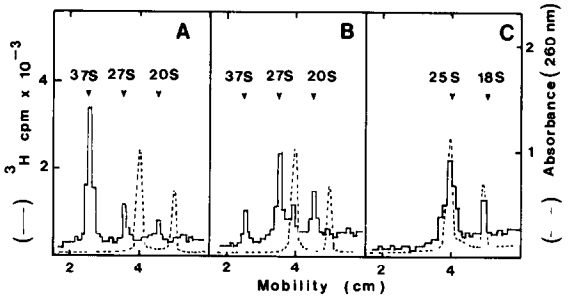

Fig.3. Processing of rRNA in the presence of SPL. VY481 cells were labelled with 30 μCi/ml [^{3}H]uracil for 60 s (A) and then chased with 10 μg/ml SPL for 5 min (B) or 10 min (C). rRNA was purified and analysed by electrophoresis in agar–urea gels.

An eventual post-transcriptional effect of SPL on the processing of rRNA was studied in VY481 cells, pulse-labelled with [^{3}H]uracil predominantly into 37 S pre-rRNA (fig.3A). The addition of 10 μg/ml SPL at this stage blocks the transcription of rRNA, but has no effect on the processing of the 37 S primary transcript to 27 S and 20 S pre-rRNA species and further to 25 S and 18 S mature rRNA (fig.3B,C). The specific radioactivity of rRNA (cpm/50 μg RNA) remained constant after the addition of SPL, suggesting an effective chase. Complete transformation of 37 S pre-rRNA to mature rRNA molecules takes place within 10 min after the chase with SPL, an observation similar to that made in kinetic studies of rRNA synthesis in yeast [11].

The results obtained show that SPL has an inhibitory effect on the transcription of rRNA genes in yeast, while the maturation of rRNA remains unaffected.

S. cerevisiae cells have large nucleoside triphosphate pools and at present the labelling of the Ado-Met pool with methyl-labelled methionine which occurs very rapidly is the only reliable way to study rRNA biosynthesis in pulse-chase experiments [7]. However, labelling with methyl-labelled Met is poor due to the low content of methylated bases in rRNA of yeast [12]. The use of SPL permits the biosynthesis of rRNA in fragile yeast to be studied in pulse-chase experiments without limitation with respect to the specific radioactivity of the RNA samples.

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