RECESSIVE SUPPRESSION IN YEAST *SACCHAROMYCES CEREVISIAE* IS MEDIATED BY A RIBOSOMAL MUTATION

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1. Introduction

Recessive nonsense suppressors in yeast *Saccharomyces cerevisiae* were first described in [1]. Later the suppressors with similar properties were isolated [2–4].

The recessive suppressor mutations arise only in two genes: *sup*1 and *sup*2 [5,6] mapped in the right arm of the second and the fourth chromosomes, respectively [5–7]. It was found that most of these suppressor mutations are completely recessive; some strains carrying a recessive suppressor mutation are temperature sensitive (*ts*-) and do not grow at elevated temperature. These facts suggested that *sup* I and *sup*2 genes may code for proteins involved in the termination of protein synthesis [5,6]. This suggestion was indirectly confirmed by biochemical studies which demonstrated that *ts*-cells carrying recessive suppressors after exposure to non-permissive temperature accumulate 80 S ribosomes with unreleased peptidyl-tRNA [8]. However, the molecular mechanism of recessive suppression in yeast still remains poorly understood.

Here evidence is presented on the similarity between recessive suppressor mutations in *Saccharomyces cerevisiae* and *ram* mutations [9,10] in *Escherichia coli*. It is suggested that the product of *sup*1 gene is a ribosomal component (most likely ribosomal protein) which is involved both in the termination of translation and in the control of the fidelity of translation.

2. Materials and methods

Two haploid strains *Saccharomyces cerevisiae* from Peterhoff yeast genetic collection were used in the study: parent strain 125A-P2156 (α *ade*1-14 *his* 7-1 *lys*2-A12 *thr*4 *leu*2-2 where α is a mating type; all 5 genetic markers are suppressible nonsense mutations) and *ts*-revertant 4-125A-P2156 (α *ade*1-14 *his* 7-1 *leu*2-2 *sup*1^{ts} *lys*2-A12 *thr*4 where *sup*1 is the recessive mutation able to suppress mutations in the genes *ade*1, *his*7 and *leu*2). The characteristics of the revertants used for the determination of codon specificity of suppression in vivo are given in table 1.

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Table 1
Prototrophy due to recessive suppression in parent strain 125A-P2156 (α *ade*1-14 *his*7-1 *lys*2-A12 *thr*4 *leu*2-2)

Total number of Ade$^+$His$^+$ revertants analyzed	Number of Ade$^+$His$^+$ revertants which simultaneously exhibit prototrophy in				
	Leu$^+$	Thr$^+$	Thr$^+$Leu$^+$	Lys$^+$Leu$^+$	Lys$^+$Thr$^+$Leu$^+$
130	99	2	22	2	5

*his*7-1, *leu*2-2, ochre mutations; *lys*2-A12, amber mutation; *thr*4, opal mutation; *ade*1-14, non-identified nonsense mutation

[11]. Cells grown under permissive conditions were used in all experiments. Post-mitochondrial extracts and ribosomes were prepared as in [8]. As the source of translational factors, tRNA and aminoacyl-tRNA synthetases, pH 5 fraction isolated from the parent strain were used throughout.

2.1. *Poly(U)-directed cell-free system of protein synthesis*

The reaction mixture for poly(U)-directed incorporation of phenylalanine and leucine contained, in 200 μl: 50 mM Tris–HCl (pH 7.6), 0.5 mM ATP, 0.15 mM GTP, 6.25 mM phosphoenolpyruvate, 5 μg phosphoenolpyruvate kinase, 0.3 mM spermidine, 50 mM KCl, 1 mM dithiothreitol, 8–20 mM $MgCl_2$, 3 A_{260} units of ribosomes, 100 μg pH 5 fraction, 1 μmol [^{14}C]phenylalanine (spec. act. 531 mCi/mmol) or [^{14}C]leucine (spec. act. 342 mCi/mmol), 100 μg poly(U) and the mixture of 19 unlabelled amino acids at 3.1×10^{-6} M each. The samples were incubated for 60 min at 20°C. In the preliminary experiments it was shown that during this time interval the incorporation of both leucine and phenylalanine was linear. Incorporation was terminated by addition of 2 ml 5% trichloroacetic acid (TCA) to each incubation mixture followed by 10 min incubation at 90°C. The precipitates were transferred to Millipore filters (0.45 μm). Filters were washed twice with 5% TCA and counted in a toluene scintillator. Absolute amounts of phenylalanine and leucine incorporated were determined. As a typical value, 70–75 pmol phenylalanine were incorporated/incubation mixture.

2.2. *Sucrose gradient centrifugation*

20–25 A_{260} units of post-mitochondrial extracts were layered onto a 7–47% (w/v) sucrose gradient and centrifuged in SW-27 rotor in Beckman L265 B Ultracentrifuge at 4°C. Gradients were analyzed by monitoring A_{260} in 0.2 ml flowcell of Perkin Elmer 304 Spectrophotometer.

3. Results

An important characteristic of any nonsense suppressor related to the degree of translational ambiguity of a strain is its codon specificity, i.e., the ability to suppress various types of nonsense mutations [2].

To study codon specificity of recessive nonsense suppressors of *Saccharomyces cerevisiae* a revertant with recessive suppressor effective towards only one type of nonsense codon was isolated and its ability to suppress two other types of nonsense codons was tested. The ability of recessive suppressors to act upon different types of nonsense mutations was revealed by the prototrophy of arising revertants (table 1).

From these results it is evident that in several suppressor-carrying strains all 3 types of nonsense mutations are suppressed. It can therefore be concluded that recessive nonsense suppression in yeast *Saccharomyces cerevisiae* is not specific towards a single type of nonsense codon and its likely mechanism may be an increased ambiguity of translation.

To measure in vitro the level of translational ambiguity a poly(U)-directed cell-free system of protein synthesis is usually used. Here the level of misreading for ribosomes from parent strain 125A-P2156 and revertant strain 4-125A-P2156 was determined by measuring misincorporation of leucine with poly(U)-template (UU-pyrimidine codes for phenylalanine whereas UU-purine and CUN code for leucine). At optimal conditions of phenylalanine incorporation the level of misreading in yeast cell-free system is rather low [12]. Therefore, various agents which increase misincorporation of amino acids are frequently added to the system to obtain a more reliable estimate of translational errors. Thus, the fidelity of translation is affected by the addition of polyamines [13], ethanol [14] and aminoglycoside antibiotics [15,16]. The ambiguity of translation also depends on the magnesium concentration for both prokaryotic and eukaryotic ribosomes [12,13]. In this study translational errors were estimated at elevated [Mg^{2+}]. The data on misreading expressed as Leu/Phe ratio at different [Mg^{2+}] are presented in fig.1. It is evident that:

(1) Both for parent and revertant ribosomes the ratio Leu/Phe incorporation increases over 8–20 mM Mg^{2+};
(2) At all the [Mg^{2+}] tested the level of ambiguity for revertant ribosomes is higher than for parent strain ribosomes.

To estimate the reproducibility of differences in ambiguity for parent and revertant ribosomes the ratio Leu/Phe was measured at fixed 16 mM Mg^{2+} in several independent experiments. The data presented in table 2 show that at 16 mM Mg^{2+} the differences in ambiguity for parent and revertant systems are statistically significant. It is pertinent to emphasize that

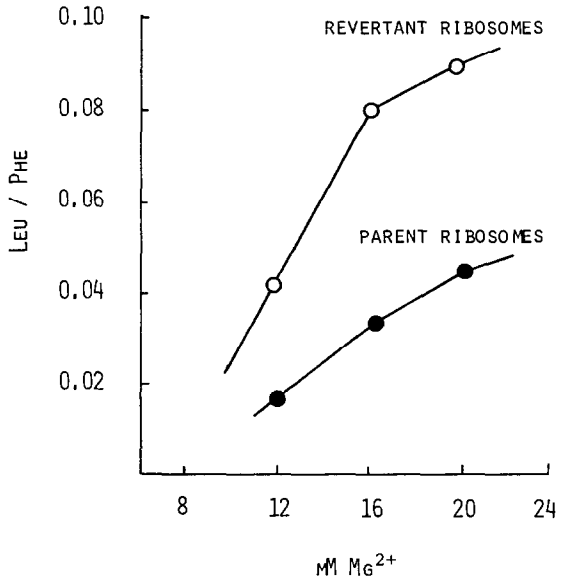

Fig.1. Ambiguity of translation versus [Mg^{2+}] in poly(U)-directed system of protein synthesis for parent and revertant ribosomes.

pH 5 fraction containing soluble translational factors and tRNA was isolated from the parent strain and therefore cannot be responsible for the different level of misreading. The pretreatment of ribosomes with high salt buffer (0.5 M KCl) which removes ribosome-bound translational factors did not affect the differences in ambiguity between parent and revertant ribosomes (data not shown).

The cells of recessive suppressor strain 4-125A-P2156 have a higher level of ribosomal subunits compared to the parent strain (fig.2a). This difference is preserved in the presence of the inhibitor of initiation, NaN_3 (fig.2b), or of elongation, cycloheximide

Table 2
Ambiguity of translation for parent and revertant ribosomes at 16 mM Mg^{2+}

Source of ribosomes	Leu/Phe[a]
Parent strain 125A-P2156	0.032 ± 0.003
ts-revertant 4-125A-P2156	0.060 ± 0.009

[a] The value Leu/Phe is expressed as means ± SEM and differ for parent and revertant strains with probability >95% both by paired *t*-tests and Wilconson criteria. Six ribosomal preparations isolated from independently inoculated and grown cell cultures were used for determination of Leu/Phe ratio for each strain

(fig.2c). Since the level of ribosomal subunits in revertant cells remains high when the translational cycle is arrested at different points it is likely that the elevated level of subunits in recessive suppressor strain is the result of the altered dissociability of 80 S ribosomes into subunits.

4. Discussion

In [8] the difference was found in the functional properties of ribosomes from a recessive suppressor

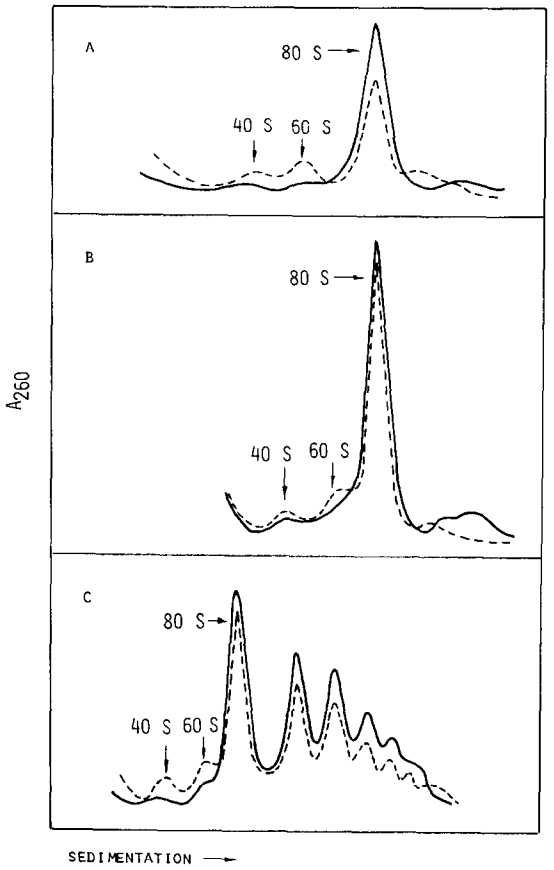

Fig.2. Sucrose gradient centrifugation of post-mitochondrial extracts from parent (——) and revertant (– – –) strains of *Saccharomyces cerevisiae*: (a) cells were incubated in the absence of any inhibitor. Gradients were centrifuged for 14 h at 20 000 rev./min; (b) NaN_3 (1 mM) was added 15 min prior to harvesting. Gradients were centrifuged for 14 h at 20 000 rev./min, (c) cycloheximide (30 mM) was added 5 min prior to harvesting. Gradients were centrifuged for 7 h at 23 000 rev./min.

and parent strains in a poly(U)-directed system of protein synthesis. However, these differences appeared only after the incubation of revertant cells at non-permissive temperature and were conditioned by the presence of unreleased polypeptides bound to 60 S subunit. From these data it was suggested that the difference between the functional properties of parent and revertant ribosomes may be a result of ribosomal mutation; however, mutational alteration of a soluble factor of protein synthesis (e.g., termination factor(s)) could not be ruled out by these results.

Here the evidence favours the ribosomal nature of the recessive suppressor mutations. This conclusion is based on the omnipotent properties of recessive suppressors, the high level of in vitro misreading by suppressor strain ribosomes and the altered dissociability of ribosomes from suppressors.

Taking into account the conclusion on the protein nature of a mutationally altered component [6,11] and the present data one can suggest that the product of *sup*1 gene is a ribosomal protein. This ribosomal protein may be involved in two functions:

1. The control of the fidelity of translation and the termination of protein synthesis. The suggestion that ribosomal protein is mutationally altered in suppressor strains is indirectly confirmed by the apparent similarity of *Saccharomyces cerevisiae* recessive suppressors and *ram* mutants of *Escherichia coli* (in particular, the omnipotency of suppression and high level of ribosomal ambiguity in vitro) [10,15].
2. The altered dissociability of ribosomes from the revertant also points to the ribosomal nature of recessive suppressor mutation. Recently, several mutants with an altered level of one or both ribosomal subunits were described: the altered level of subunits in these mutants was found to be a result of mutational alteration of ribosomal protein [17,18].

Thus, our results demonstrate the existence of ribosomal mutants in yeast *Saccharomyces cerevisiae* with altered discriminating ability. Similar data were obtained for another eukaryote, *Podospora anserina* [19]. The finding of ribosomal mutants for the simplest eukaryotes may be important for a better understanding of the function of individual ribosomal components and of the organization of eukaryotic ribosome.

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