RECESSIVE NONSENSE-SUPPRESSION IN YEAST: FURTHER CHARACTERIZATION OF A DEFECT IN TRANSLATION

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1. Introduction

In previous papers from this laboratory [1,2] it was shown that the temperature-sensitive (*ts*-) strain of yeast *Saccharomyces cerevisiae* harboring a recessive nonsense-suppressor mutation in the sup2 gene carries a defect in the mechanism of translation. This alteration leads to a suppression effect at permissive conditions (25°C) and to a rapid cessation of protein synthesis at restrictive temperature (36°C). From preliminary data a suggestion was made that it is the mechanism of peptide chain termination that is impaired in the suppressor strain. In this paper we demonstrate that at 36°C *ts*-revertant accumulates 80S ribosomes which do carry unreleased peptidyl-tRNA.

2. Materials and methods

Two haploid strains *Saccharomyces cerevisiae* from the Peterhoff yeast genetic collection were used in this study: parent strain (3-P219, an ade1-14 hisX lys2-A12, all three genetic markers are suppressible nonsense mutations) and *ts*-revertant (14-3-P219, an ade1-14 hisX lys2-A12 sup2 where sup2 is recessive suppressor mutation). Growing conditions for these strains and composition of media were published earlier [2,3]. In all experiments disintegration of cells was done in an SCP disintegrator (LKB, Sweden) with 0.5 mm glass beads. The method of Zeijst et al. [4] was followed with some modifications for isolation of ribosomal subunits and 'run-off' 80S ribosomes.

3. Results

An inhibition of protein synthesis in *ts*-revertant strain 14-3-P219 following the shift from permissive to restrictive temperature can occur due to a number of lesions in translational mechanism many of which would eventually result in polysome breakdown. We compared the state of polysomes in parent and *ts*-revertant strains at different time intervals after shifting cultures from 25°C to 36°C. The results are shown in fig.1. In contrast to the parent strain, definite changes in polysomal pattern of the *ts*-revertant occur after the shift. As shown in fig.1 the decrease in the heavy polysome content is accompanied by an increase of monosomes. These changes in polysomal profile are first registered somewhere between 5 and 10 min after the shift to 36°C. In 30 min more than 90% of ribosomes in *ts*-revertant cells are present as 80S ribosomes. Thus, inhibition of protein synthesis in *ts*-revertant cells at restrictive temperature occurs because of polysome decay under non-permissive conditions.

In a search for a mutant component of the translational machinery responsible for suppression and thermosensitivity of the suppressor strain, it is important to know the nature of the 80S particles accumulating in *ts*-revertants in restrictive conditions. The increase in 80S ribosome content may be a reflection of their inability to dissociate into subunits due to the presence of unreleased peptidyl-tRNA. This explanation was indirectly supported by our data on the radioactivity of peptides associated with ribosomes from *ts*-revertant cells at 36°C [2]. It could be expected that ribosomes

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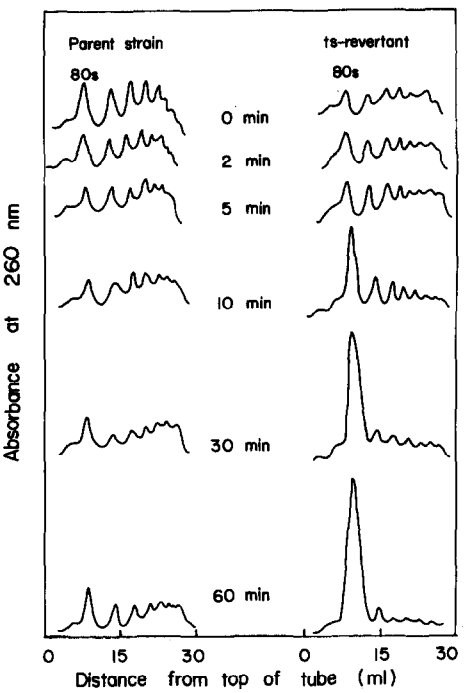

Fig.1. Kinetics of polyribosome decay in the *ts*-revertant at 36° C. Cells grown aerobically at 25°C were shifted to 36°C and incubation continued for 2,5,10,30 and 60 min. Cycloheximide was added to each culture, the temperature lowered by addition of ice into the flasks and the cells were collected on filters. Cells were suspended in 0.05 M Tris-HCl pH 7.6, 10 mM $MgCl_2$, 25 mM KCl, 4 mM dithiothreitol, disintegrated and suspensions centrifuged at 15 000 *g* for 20 min. Aliquots of supernatants were layered on sucrose gradients for polysome analysis [10]. The left part of the Figure shows the polyribosome pattern in the parent strain under strictly identical conditions.

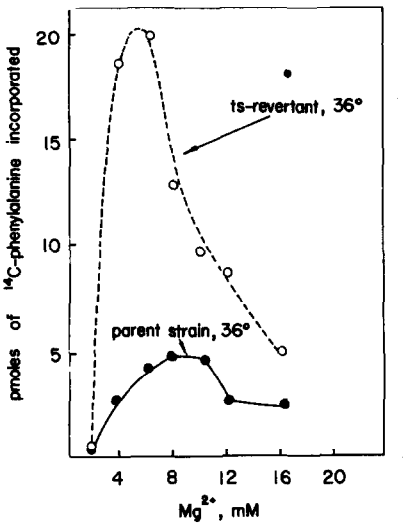

Fig.2. Poly U-dependent [^{14}C]phenylalanine incorporation in S-30 fractions from parent and *ts*-revertant strains versus magnesium concentration. For preparation of S-30 extracts, cultures growing at 25°C were transferred to 36°C and incubated for an additional 30 min. The procedure of Schlanger et al. [11] was essentially followed in the preparation of S-30 fractions and in the study of cell-free [^{14}C]phenylalanine incorporation. Incubation was carried out of 25°C for 30 min. Each assay contained 0.2 μCi of [^{14}C]phenylalanine (specific activity 477 μCi/mmole) and 500 μg of protein of corresponding S-30 fraction.

carrying peptidyl-tRNA would differ in some way from free 80S ribosomes in poly U-dependent [^{14}C]phenylalanine incorporation. To test this suggestion we checked the Mg^{2+}-dependence of phenylalanine incorporation in S-30 extracts from parent and *ts*-revertant cultures preincubated at 36°C. The results are shown in fig.2. Two conclusions are apparent from this figure: (i). The rate of [^{14}C]phenylalanine incorporation is much higher in the S-30 extract from the *ts*-revertant; (ii) maximal incorporation in the S-30 fraction from the *ts*-revertant is registered at lower magnesium concentration than in the extract from the parent strain.

Studying endogeneous incorporation of [^{14}C]amino acids in cell-free systems from the *ts*-revertant and the parent strain we have earlier demonstrated [2] that thermosensitivity of soluble factors from both strains is similar. Hence it was plausible to suggest that the shift in Mg^{2+} concentration optimum of poly U-dependent phenylalanine incorporation and the higher rate of incorporation in the S-30 fraction from the *ts*-revertant are due to differences in ribosomes. This suggestion was confirmed in the study of poly U-dependent [^{14}C]phenylalanine incorporation by 80S ribosomes reconstituted from purified 60S and 40S subunits (in homologous and hybrid cell-free systems) from the *ts*-revertant and the parent strains. As a source of soluble factors the pH 5 fraction from the parent strain was used in all assays. The results of this experiment (fig.3) demonstrate that the differences in Mg^{2+} concentration optimum and the levels of [^{14}C]phenylalanine incorporation between *ts*-revertant and parent strains are preserved in the purified system. Moreover, when hybrid systems are

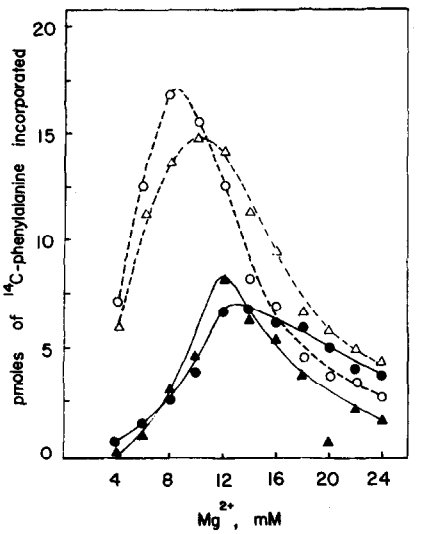

Fig.3. Activity of 80S ribosomes reconstituted from *ts*-revertant and parent subunits in poly U-dependent [^{14}C]phenylalanine incorporation. Parental 60S and 40S particles were isolated from 'run-off' 80S ribosomes. *ts*-revertant cells incubated for 30 min at 36°C served for the isolation of revertant ribosomal subunits. Cell-free mixtures [4] were incubated at 25°C for 30 min. The parent pH 5 fraction was used as a source of soluble factors and tRNA. Hot TCA-insoluble incorporation was measured by a standard technique on Millipore filters. Each assay contained 0.1 μCi of [^{14}C]phenylalanine, 1.0 unit A_{260} of 60S particles and 0.5 unit of 40S particles derived either from the same strain (controls) or both strains (hybrids). (-○- - -○-) 40S and 60S from *ts*-revertant (-△- - -△-) 40S from parent, 60S from *ts*-revertant (—●—●—) 40S and 60S from parent strain (—▲—▲—) 40S from *ts*-revertant, 60S from parent strain.

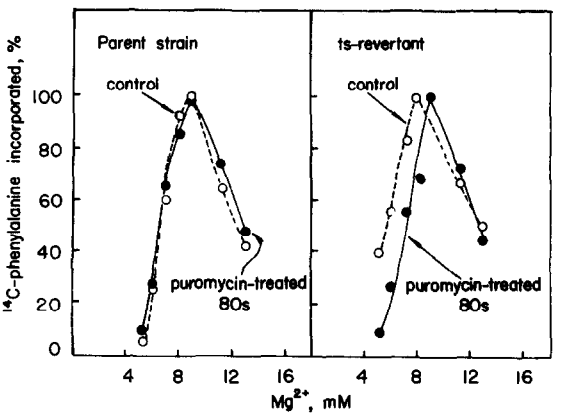

Fig.4. Effect of puromycin treatment of 80S ribosomes on poly U-dependent [^{14}C]phenylalanine incorporation. Parent and *ts*-revertant cultures grown at 25°C were transferred to 36°C and incubation continued for 30 min. Before harvesting NaN_3 was added to the parent culture, cells were collected, disintegrated and ribosomes prepared by differential centrifugation. Ribosomes were suspended in buffer (see legend for fig.1), cleared from aggregates, and incubated with puromycin (50 μg/ml) Control and puromycin-treated samples were centrifuged through 0.6 M sucrose to pellet ribosomes. Resuspended ribosomes were used in experiments on poly U-dependent [^{14}C]phenylalanine incorporation.

studied it becomes evident that it is the 60S subunit of the *ts*-revertant that is responsible for these differences.

Since parent 60S particles were isolated from 'run-off' ribosomes and were free from peptidyl-tRNA it was suggested that high poly U-dependent [^{14}C]phenylalanine incorporation at low magnesium concentration, by ribosomes from 36°C-treated *ts*-revertant cells might be due to peptidyl residues present in the 60S particles. If this was so, the differences in [^{14}C]phenylalanine incorporation should be abolished after treatment of *ts*-revertant ribosomes with puromycin. The effect of puromycin on poly U-dependent [^{14}C]phenylalanine incorporation by parent and *ts*-revertant ribosomes is shown in fig.4. It is seen that puromycin has no effect on Mg^{2+} concentration dependence of [^{14}C]phenylalanine incorporation by parent ribosomes and shifts the Mg^{2+} concentration optimum of incorporation to higher valnes in *ts*-revertant ribosomes.

4. Discussion

In our previous studies of translation in *ts*-revertant 14-3P219 we were unable to reveal the differences in polysomal pattern between parent and *ts*-revertant strains after the shift to 36°C. This was apparently due to partial run-off during spheroplast isolation and lysis in spite of the presence of cycloheximide. Direct disintegration of cells now allows one to relate the cessation of protein synthesis to polysome decay in the *ts*-revertant strain at 36°C.

The study of 80S ribosomes accumulating in *ts*-revertant at 36°C showed that these ribosomes differ from run-off ribosomes in magnesium dependence of [^{14}C]phenylalanine incorporation in the presence of poly U, i.e. at low magnesium concentrations they

incorporate more [^{14}C]phenylalanine. This could result from (i) decreased dependency of 80S ribosomes from the 36°C-treated *ts*-revertant on initiation factors [5] and (ii) a different mode of [^{14}C]phenylalanine incorporation by *ts*-revertant ribosomes at low magnesium concentration. The experiments with hybrid ribosomes demonstrated that when 60S subunits from a 36°C-treated *ts*-revertant culture are present in hybrid ribosomes a higher [^{14}C]phenylalanine incorporation at low magnesium concentration is observed.

The localization of differences in ribosomes at the level of the 60S subunit and the fact that puromycin abolishes these differences suggest that high [^{14}C]phenylalanine incorporation at low magnesium concentration is directly related to the presence of peptidyl-tRNA bound to 60S subunits from the *ts*-revertant strain.

In cell-free systems derived from rat muscle ribosomes [6], *Neurospora crassa* [7] and yeast *Saccharomyces carlsbergensis* [8,9], programmed with polyuridylic acid, it was found that at low Mg^{2+} concentrations (7–8 mM) poly U is capable of supporting phenylalanine incorporation into the C-terminal position of preexisting nascent polypeptide. It is reasonable to believe that a similar type of [^{14}C]phenylalanine incorporation takes place in the presence of ribosomes from the *ts*-revertant strain incubated at 36°C.

The presence of unreleased peptide in ribosomes of the *ts*-revertant in restrictive conditions demonstrates that recessive suppression in yeast is likely to involve mutational change in the mechanism of termination. Exact localization of the lesion requires further work.

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