RECESSIVE SUPPRESSION AND PROTEIN SYNTHESIS IN YEAST

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1. Introduction

In addition to well-known dominant suppressors [1,2] in *Saccharomyces cerevisiae* two genes [s_1 and s_2] have been found in which recessive suppressor mutations occur [3]. Like dominant recessive suppressor mutants they are able to translate nonsense-mutations [4]. Some of recessive suppressor mutations result not only in suppression of nonsense-mutations but also in temperature sensitivity, i.e., they are lethal at restrictive temperature.

The examination of genetic behaviour of these mutants have suggested that s_1 and s_2 genes may control proteins participating in termination of polypeptide chain synthesis on ribosomes [3].

To test this hypothesis we have studied protein synthesis in temperature-sensitive (ts^-) and temperature-resistant (tr^-) recessive suppressor mutants at permissive (25°C) and restrictive (36°C) temperatures. It was demonstrated that after a shift to restrictive temperature the mechanism of peptide chain termination becomes defective in a temperature-sensitive strain of yeast carrying recessive suppressor mutation in s_2 gene.

2. Materials and methods

The yeast strains used in this work were haploid *S. cerevisiae* from Peterhoff genetic stock. The parent is designated 3-P219 (a ade_{1-14} his_x lys_{1-A12}) (all three genetic markers are suppressable nonsense-mutations). Two revertants of the parent strain are designated as 12-3-P219 – *tr*-suppressor mutant and 14-3-P219 – *ts*-supptessor mutant. Both mutants are able to synthesize adenine and histidine. These mutants were isolated as recessive revertants after ultraviolet-irradiation of the parent 3-P219. The following abbreviations for genetic markers are used: (a) indicates mating type; (ade, his, lys) indicate inability to synthesize adenine, histidine and lysine, respectively.

Composition of media and growing of cultures were published earlier [5]. Procedure proposed by Duell et al. [6] was followed with some modifications to prepare spheroplasts. Polysomes were isolated from spheroplasts by a modified Hartwell method [7,8]. The composition of yeast cell-free system was essentially that of Richter et al. [9]. tRNA was isolated from parent and *ts*-revertant strains according to Holley [10].

Soluble factors for yeast cell-free system were prepared by removal of nucleic acids from 105 000 *g* supernatant of cells by streptomycin and subsequent isolation of protein fraction precipitated between 30 and 70% saturation with $(NH_4)_2SO_4$.

3. Results

If *ts*-revertants do not grow at 36°C due to a lesion in the translation system a decrease in the total rate of protein synthesis may be expected to occur. To test this suggestion the examination of the kinetics of [^{14}C] amino acid incorporation into proteins in strains 3-P219, its *tr*- and *ts*-revertants has been carried out at 25°C and 36°C (fig. 1,A,B,C). While parent strain and *tr*-suppressor reveal some stimulation of protein synthesis at 36°C as compared to 25°C, the rate of [^{14}C] amino acid incorporation into proteins

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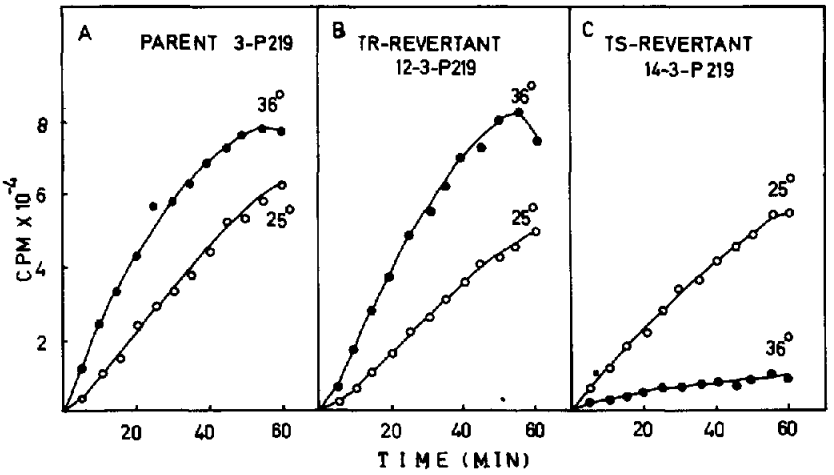

Fig. 1. Kinetics of protein synthesis at 25°C and 36°C in parent strain 3-P219, *tr*-revertant 12-3-P219 and *ts*-revertant 14-3-P219: (A) parent strain, 3-P219; (B) *tr*-revertant, 12-3-P219; (C) *ts*-revertant, 14-3-P219. Cells grown aerobically at 25°C on synthetic medium were harvested in the middle of the exponential phase and resuspended in fresh medium (absorbance 1.0 at 540 nm, 1 mm cell, FEC-56M photometer). To suspensions preincubated at 25° or 36°C for 60 min the mixture of ^{14}C-labelled amino acids was added (3 μCi/ml) and incubation continued. At intervals samples were taken from suspensions into 10% trichloroacetic acid. Nucleic acids were hydrolysed and radioactivity incorporated into protein was determined on Millipore filters with a toluene scintillator.

by *ts*-revertant at restrictive temperatue is very low. It can be concluded that temperature sensitivity of *ts*-recessive suppressor is indeed related to some alteration in the translation. At 25°C this defect may not be apparent or expressed as the effect of suppression. Using restrictive temperature to amplify phenotypic effect of suppressor mutation we made an attempt to locate a thermolabile step of protein synthesis by examining the temperature sensitivity of the separate events of translation in *ts*-revertant.

The inhibition of protein synthesis in *ts*-revertant at 36°C may occur because of breakdown of polysomes to monosomes due to temperature sensitivity in the initiation of polypeptide chains. This was tested by sedimentation study of polysomes isolated from the parent strain and *ts*-revertant cells previously incubated at 36°C for 60 min. Fig. 2 shows that the sedimentation profiles of polysomes from both strains are similar: no increase in the content of 80 S ribosomes or their subunits is found in *ts*-revertant cells at 36°C compared to parent 3-P219.

Temperature sensitivity of the elongation step in parent 3-P219 and *ts*-revertant strain was studied in vitro employing a cell-free system with polysomes and soluble factors isolated from these strains. Although in vitro release of nascent polypeptides synthesized by the system is rather limited, a linear rate of amino acid incorporation into polypeptides was observed for 60 min. Thus the system is suitable to study the temperature sensitivity of elongation and initiation of polypeptides. The results of this comparative study are shown in fig. 3. As these kinetic data demonstrate both polysomes and soluble factors from parent and *ts*-revertant exhibit the same temperature sensitivity. Thus *ts*-lesion in *ts*-revertant 14-3-P219 is not related to the initiation or elongation steps of protein synthesis.

To study temperature sensitivity of the elongation and termination steps of translation in *ts*-revertant we went back to experiments on the kinetics of protein synthesis at 25°C and 36°C in vivo. In order to prevent the formation of new polysomes NaF was added to cell suspensions [11]. The inhibition of amino acid incorporation into proteins of both parent and *ts*-revertant cells is observed in the presence of sodium fluoride (fig. 4). Difference in sensitivity of the translation mechanism of the strains studied towards NaF is evident even at 25°C: inhibition of protein synthesis is more profound in *ts*-revertant (fig. 4,A,B). At 36°C in the presence of NaF proteins are synthesized at a decreased but linear rate for 15 min by parent cells. Under the same conditions the rate of amino acid incorporation into proteins by *ts*-revertant is negligible (fig. 4,C,D).

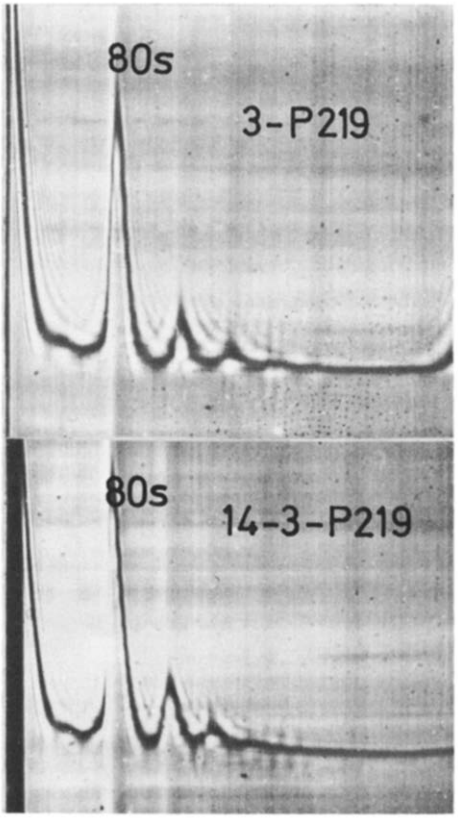

Fig. 2. Sedimentation pattern of polysomes from parent 3-P219 and *ts*-revertant 14-3-P219 cells incubated for 60 min at 36°C. Cells grown aerobically at 25°C on YEPD medium were shifted to 36°C and incubation continued for an additional 60 min. Cycloheximide was added to cultures (50 μg/ml), cells were collected on filters and converted into spheroplasts in the presence of cycloheximide. Spheroplasts were lysed [8] and lysates were analysed on a Spinco model E analytical centrifuge at 25°C and 24 630 rev/min Photographs were taken with Schlieren optics 12 min after this speed was attained.

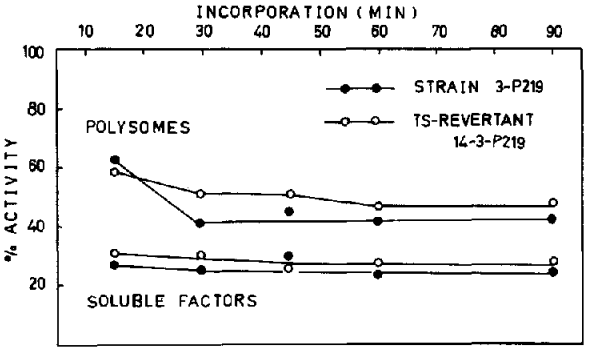

Fig. 3. Temperature sensitivity of polysomes and soluble factors from parent strain 3-P219 and *ts*-revertant 14-3-P219 in a cell-free system. Polysomes and soluble factors were heated for 20 min at 45°C before addition to a cell-free system. The following reaction mixtures were made for each strain: 1) heated polysomes; 2) heated soluble factors; 3) all native components and homologous tRNA 0.5 μCi of ^{14}C-labelled amino acid mixture (0.025 μCi of each amino acid) was added to each tube. Samples were withdrawn at intervals for determination of radioactivity incorporated into proteins. Percentage of residual activity was calculated as the ratio of radioactivity of the sample with heated component to that of the control at corresponding incubation time.

Following 30 min incubation of parent and *ts*-revertant cultures with radioactive amino acids in the presence of NaF, sucrose gradient analysis of polysomes from these cells was carried out. At 25°C the distribution of label along the gradient in both strains is almost the same (fig. 5,A,C). Some label is present in the heavy polysome region, part of the radioactivity is bound to 80 S particles and most of the label is in the released polypeptides in the upper part of the gradients. At 36°C the differences in the pattern of polysome labelling between strain 3-P219 and *ts*-revertant become apparent (fig. 5,B.D). By 30 min incubation of parent cells with [^{14}C] amino acid most of label left the polysome region, some label is found in the 80 S zone and most is in the released polypeptides. Radioactivity profile of polysomes from *ts*-revertant is different: no peak being seen in 80 S region.

4. Discussion

The examination of the rates of protein synthesis in parent strain of yeast 3-P219, *tr*-revertant 12-3-P219 and *ts*-revertant 14-3-P219 have shown that *ts*-revertant carries an alteration in the translation system resulting in the thermolability of protein synthesis at restrictive temperature. The analysis of initiation and elongation steps of translation revealed no differences in temperature sensitivity between parent and *ts*-revertant strains. The polysome profile in both cultures was similar after incubation of cells at 36°C. In cell-free systems temperature sensitivity of

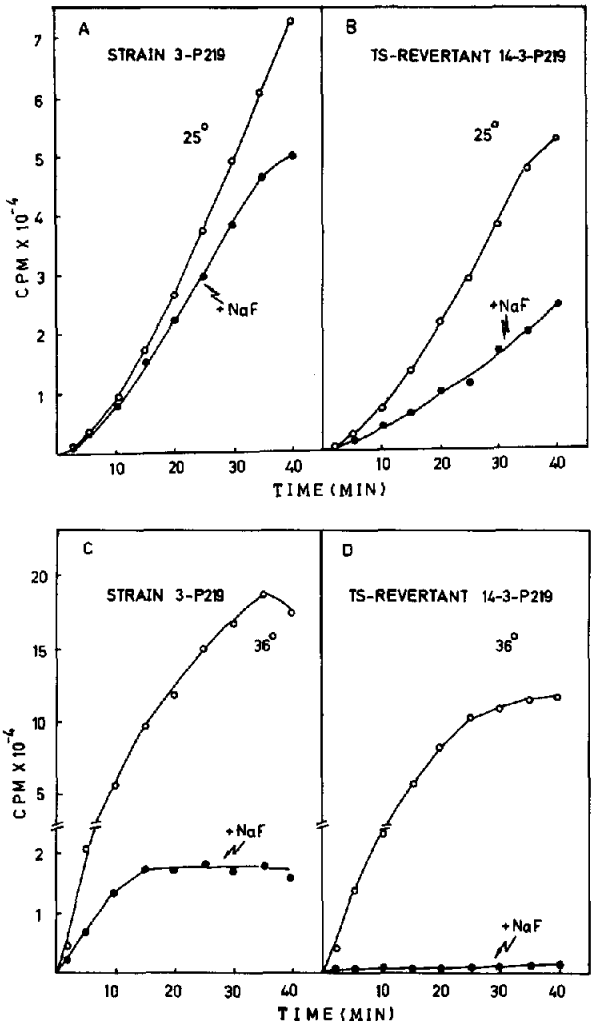

Fig. 4. Kinetics of protein synthesis at 25° and 36°C in parent strain 3-P219 and *ts*-revertant 14-3-P219 in the presence of NaF: (A) parent strain, 3-P219, 25°C; (B) *ts*-revertant, 14-3-P219, 25°C; (C) parent strain 3-P219, 36°C; (D) *ts*-revertant 14-3-P219, 36°C. Cells grown aerobically at 25°C on synthetic medium were harvested in the middle of exponential phase by centrifugation and resuspended in fresh medium (absorbance 1.0 at 540 nm). Sodium fluoride was added to aliquots of suspensions (final concentration 0.05M) and tubes were placed at 25°C or 36°C. In 5 min 0.5 μCi of ^{14}C-labelled amino acid mixture (0.025 μCi of each amino acid) was added to each suspension and incubation continued. Samples were taken from suspensions at various intervals for determination of protein radioactivity.

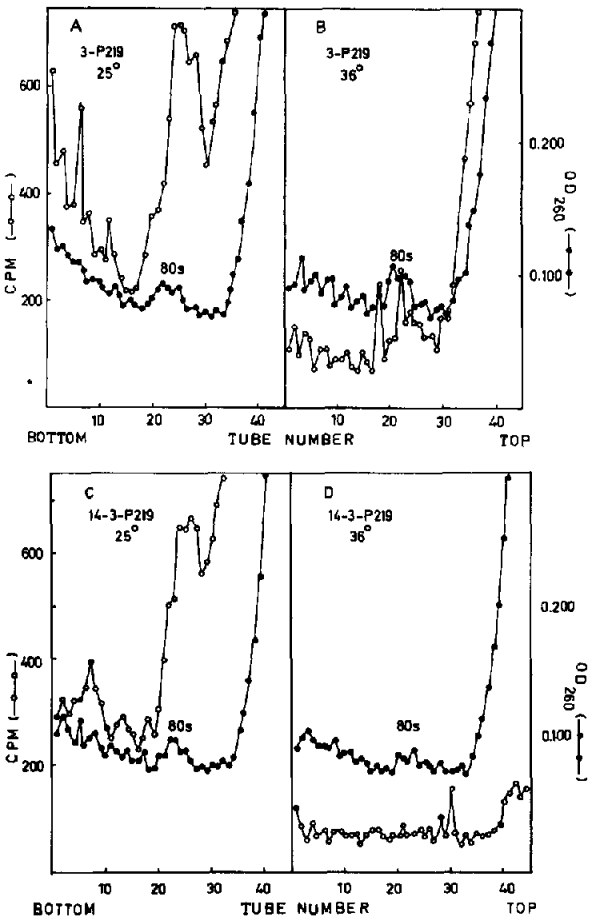

Fig. 5. Sucrose gradient analysis of parent 3-P219 and *ts*-revertant 14-3-P219 lysates after incorporation of ^{14}C-labelled amino acids at 25°C and 36°C in the presence of NaF:(A) parent strain 3-P219, 25°C; (B) parent strain 3-P219, 36°C; (C) *ts*-revertant 14-3-P219, 25°C; (D) *ts*-revertant 14-3-P219, 36°C. Incorporation of ^{14}C-labelled amino acids into proteins (see legend to fig. 4) was stopped after 30 min of incubation by addition of cycloheximide (100 μg/ml) and cooling of tubes on ice. Cells were collected on filters and used for the preparation of spheroplasts. Spheroplasts were lysed, debris centrifuged down and supernatants were layered on the top of 7–47% sucrose gradients for polysome analysis [12]. Gradients were centrifuged at 25 000 rev/min in a Spinco SW-27 rotor for 7 hr. Gradients were analysed for absorption at 260 nm and radioactivity of proteins by standard methods.

polysomes and soluble factors from parent and *ts*-revertant was also the same.

The only difference between parent strain and *ts*-revertant at the level of translation was found in experiments with NaF as inhibitor of initiation. *ts*-Revertant was shown to be more sensitive to NaF especially at restrictive temperature. Two explanations for high sensitivity of *ts*-revertant may be suggested:

1) NaF acts on the translation step carrying an alteration due to suppressor mutation. At 25°C this defect is revealed due to the presence of NaF. At 36°C, NaF and thermolability effects may be additive which results in almost complete cessation of translation.

2) NaF and suppression act on different translation steps. When initiation is inhibited by NaF the cessation of translation at 36°C in *ts*-revertant is caused by a defect in elongation or termination steps. The same effect although less pronounced is also observed at 25°C.

Since initiation seems to be unaltered in *ts*-revertant at 36°C the first explanation of high sensitivity of *ts*-revertant to NaF is ruled out. The fact that *ts*-revertant carries no defect in elongation suggests that thermolability of *ts*-revertant is caused by an alteration in the termination of translation. In fact, if termination is stopped at restrictive temperature all elongating ribosomes of a polysome would cease their movement along the mRNA tape; thus no run-off ribosomes with completed polypeptides would be found in extracts of such cells. This was indeed observed in the experiment presented in fig. 5. While at 36°C, with added NaF, the amino acid label moves from polysomes into the 80 S region and is further released with polypeptides in parent cells, no peak of radioactivity bound to 80 S ribosomes is seen under the same conditions in *ts*-revertant. Such polysomes though structurally normal are not active functionally.

Our results demonstrate that recessive super-suppression in yeast is apparently similar to suppression of UAA and UGA codons in *E. coli* [13–15] and recessive UGA suppression in *Salmonella typhimurium* [16].

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