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Inaccurate protein synthesis in a mutant of *Salmonella typhimurium* defective in transfer RNA pseudouridylation

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Protein synthesis was studied comparatively in a wild-type strain of *Salmonella typhimurium* and in *hisT* mutant cells defective in the pseudouridylation of transfer RNA. From a quantitative point of view, no significant difference between the two types of strain was observed when measuring the rate of protein synthesis during either exponential growth or starvation for histidine. In contrast, the qualitative analysis of proteins by two-dimensional gel electrophoresis showed that histidine-starved *hisT* cells mistranslate the genetic program at a higher frequency than exponentially growing *hisT* cells or either starved or unstarved *hisT*$^+$ cells.

Pseudouridylation; *hisT* mutation; Mistranslation; *(Salmonella typhimurium)*

1. INTRODUCTION

In *Salmonella typhimurium*, the production of the enzymes of the histidine operon is affected by mutations in any of six different genetic loci [1]. One of these is the structural gene *hisT* encoding the enzyme pseudouridylate synthetase which catalyzes the conversion of specific uridine residues to pseudouridine during maturation of several transfer RNAs [2,3]. *hisT* mutants contain a $tRNA^{His}$ lacking two pseudouridine residues which are normally present in positions 38 and 39 of the anticodon region of the wild-type $tRNA^{His}$ [4,5]. In spite of this defect, the total amount of $tRNA^{His}$ and the in vivo ratio of charged to uncharged $tRNA^{His}$ in *hisT* mutants are identical to those measured in the wild-type [6]. In addition, $tRNA^{His}$ isolated from *hisT* mutants is normally charged and has the same affinity for histidyl-tRNA synthetase as does the modified tRNA [7]. In this context the overall rate of protein synthesis in the mutants remains comparable to that in wild-type cells, and the *hisT* gene product does not seem to be essential for the growth of bacteria [4,8].

In the present work, the effect of the *hisT* mutation on protein synthesis has been investigated from a qualitative point of view so as to determine whether the lack of $tRNA^{His}$ pseudouridylation influences the accuracy of the translation process. The occurrence of misreading has been analyzed in an otherwise isogenic pair of *hisT*$^+$/*hisT* strains of *S. typhimurium*, using two-dimensional gel electrophoresis to detect the charge heterogeneity induced by amino acid substitutions in error-containing proteins [9,10]. Since starvation of cells for a required amino acid can lead to an increase in the frequency of translational mistakes [10–12], experiments have been performed comparatively in the presence and the absence of histidine which is essential for the normal growth of the strains used. Moreover, since bacteria of stringent (*relA*$^+$) and relaxed (*relA*) genotype are known to exhibit a different sensitivity to misreading [13,14], the behavior of a *relA hisT* double mutant has been analyzed in parallel.

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2. MATERIALS AND METHODS

The *S. typhimurium* strain TA997 (*relA*$^+$, *hisT*$^+$, *aroC*, *pur*145, *thi*, *hisD*2655), its derivative strain TA1001 (*relA*$^+$, *hisT*, *aroC*, *pur*145, *thi*, *hisD*2655), and the corresponding relaxed mutant PD2 (*relA*, *hisT*, *aroC*, *pur*145, *thi*, *hisD*2655) previously described [15] were used throughout experiments.

Cells were grown at 37°C in a minimal medium containing 100 mM Tris-HCl at pH 7.4, 27 mM KCl, 37 mM NH_4Cl, 2.7 mM $MgCl_2 \cdot 6H_2O$, 0.1 mM Na_2SO_4, 0.007 mM $FeCl_3$, 0.5 mM KH_2PO_4, 0.15 mM $CaCl_2$, 10 mM glucose and 0.015 mM thiamine. Histidine and other compounds required for growth were added at a final concentration of 50 μg/ml each.

Starvation experiments were performed by transferring exponentially growing bacteria to fresh medium lacking histidine, as reported in [16].

For the measurement of protein synthesis, cultures were labeled with [^{14}C]proline (0.04 μCi and 10 μg per ml; spec. act. 250 mCi/mmol, CEA, France), and 400 μl samples were taken into 5% trichloroacetic acid. The bacterial precipitates were collected on Millipore filters and the radioactivity was counted [16].

To prepare radioactive cellular extracts in view of electrophoretic analysis, cells were first labeled for 45 min with [^{35}S]methionine (5 μCi/ml; spec. act. 1400 Ci/mmol, Amersham Centre, England), in the exponential phase of growth or during histidine starvation, and then collected by low-speed centrifugation. The pellet was suspended in a buffer containing 10 mM Tris-HCl at pH 7.4, 5 mM $MgCl_2$ and 50 μg/ml pancreatic ribonuclease, and bacteria were disrupted by repeated ultrasonic treatment. The sonicated extract was incubated for 15 min at 4°C in the presence of 50 μg/ml pancreatic deoxyribonuclease. After centrifugation for 25 min at 30000 × *g*, the supernatant (fraction S30) was collected, proteins were treated with 0.12 vol. of 3% SDS/10% β-mercaptoethanol mixture, precipitated overnight with 5 vol. of 95% acetone at −20°C, and finally centrifuged and dried under vacuum.

The O'Farrell two-dimensional gel technique [9] was used to analyze the labeled proteins of fraction S30. Separation in the first dimension was achieved by isoelectric focusing to equilibrium (10000 V·h) in pH 5–7 ampholine in a 4% acrylamide gel containing 9.5 M urea. Electrophoresis in the second dimension was performed in 12.5% acrylamide and 1% SDS (600 V·h). The amount of protein loaded on gels (20–30 μg/gel) was determined by the Bradford technique [17].

For autoradiography, the gel was incubated for 2 h at room temperature in a 7.5% acetic acid/30% methanol mixture, dried under vacuum and exposed for 2–3 days using direct-exposure DEF-5 film from Eastman Kodak Co.

3. RESULTS AND DISCUSSION

In a first series of experiments, the extent of residual protein synthesis during amino acid starvation of bacteria was measured and compared to that in exponentially growing cells. The wild-type strain TA997, the *hisT* mutant TA1001, and the relaxed derivative *hisT relA* strain PD2 were deprived separately of histidine, and the amount of radioactive proline incorporated into the acid-precipitable material was determined, in each case, at various times after the onset of starvation. The results presented in fig.1 show that, in any type of strain, the rate of residual protein synthesis during histidine starvation is reduced to the same low value of about 7% of the control. They also indicate that during normal growth in the presence of histidine, the rate of protein synthesis in the *hisT* mutants is quite similar to that measured in the wild-type strain, in agreement with other results reported previously [4,8].

A qualitative analysis of the protein content of strains TA997, TA1001 and PD2 was performed. For this, bacteria were cultured in complete medium for about 2 generations, then either maintained under normal conditions of growth or transferred to starvation medium lacking histidine; in all cases, they were labeled with radioactive methionine. Protein extracts (fraction S30) were prepared and analyzed by two-dimensional electrophoresis according to the O'Farrell gel technique [9] followed by autoradiography. In this procedure it is expected that if an amino acid erroneously incorporated into a protein differs in charge from the normal amino acid, the mistranslated protein will also differ in charge from the normal protein. Consequently, such amino acid substitutions will yield satellite protein spots aligned in series in the isofocusing dimension [9,10]. The formation of multiple 'stutter-spots' has thus been observed previously in relaxed, but not stringent, bacteria during amino acid starvation [9,10]. It has also been detected in either type of strain under treatment with error-inducing antibiotics, namely streptomycin, even in the presence of all the amino acids required for growth [12,18]. By contrast, no stuttering occurs in normally growing stringent or relaxed bacteria [9,19].

The autoradiograms presented in fig.2 show, on the one hand, that as expected no electrophoretic heterogeneity of proteins is detectable in the extracts prepared from exponentially growing cells of the wild-type strain (diagram a); diagrams b and c of the same figure show that extracts from exponential *hisT* cells give a similar electrophoretic pattern. On the other hand, a differential response is observed when bacteria are deprived of histidine: strain TA997 gives a protein pattern (diagram d)

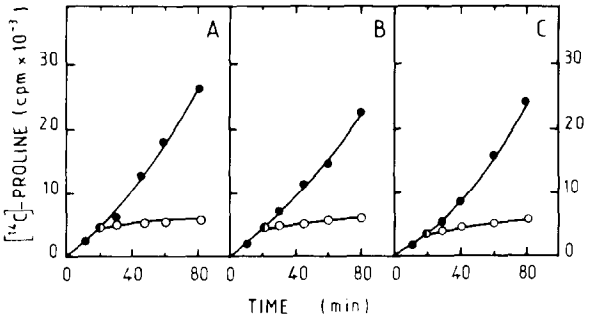

Fig.1. Protein synthesis in strains TA997, TA1001 and PD2 of *S. typhimurium*. Cells were cultured for 20 min in the presence of [^{14}C]proline then either maintained in exponential phase of growth for an additional 60 min period (●) or starved for histidine (○). Samples were withdrawn after the indicated times, precipitated with trichloroacetic acid and the radioactivity was counted. (a) Strain TA997; (b) strain TA1001; (c) strain PD2.

similar to that obtained with unstarved cells but, by striking contrast, a significant heterogeneity of several proteins is detected in mutant TA1001 (diagram e). The same charge heterogeneity is observed in the histidine-starved mutant PD2, as expected for every relaxed mutant deprived of a required amino acid [9,19]. The leftmost, most basic, spot of each series corresponds to the protein made in unstarved bacteria (shown by arrowheads), the displacement of the satellite spots being toward the acidic end of the gel. Obviously, such a shift must be due to the substitution of the basic amino acid histidine by a neutral or an acidic amino acid. In particular, the histidine codons CAU and CAC could be misread as the glutamine codons CAA and CAG, resulting in the misincorporation of a neutral amino acid. This interpretation would support the concept that misreading affects preferentially the third base of the codon [20,21].

Our results furnish evidence that *hisT* mutants mistranslate at a higher frequency than *hisT*$^+$ strains during histidine starvation. If histidine codons are actually mistaken for glutamine codons, the mistranslation must be at the level of the tRNA^{Gln} isoacceptors. Since these isoacceptors themselves contain some pseudouridine residues in their anticodon region [5], it therefore appears that undermodified tRNAs would mistranslate more easily than normal tRNAs. It could also be envisaged that the *hisT* product itself, the

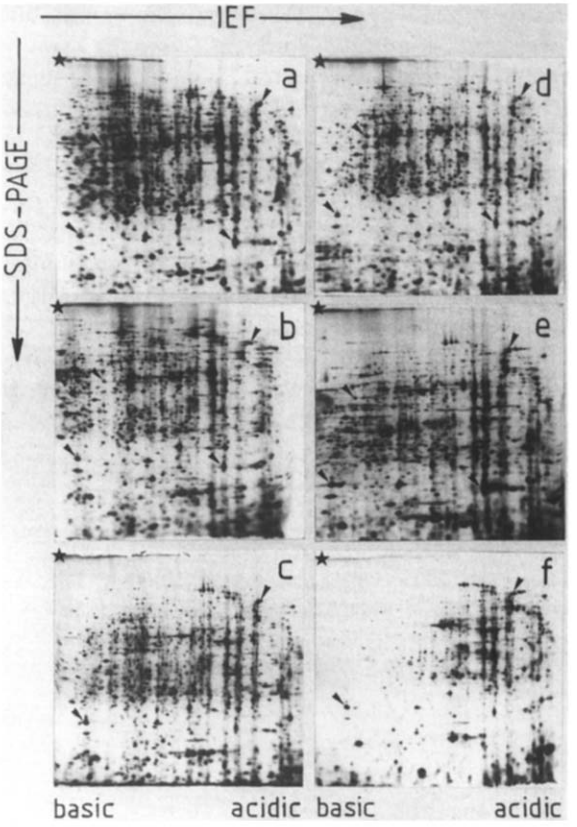

Fig.2. Two-dimensional analysis of radioactively labeled proteins. Cells of strains TA997, TA1001 and PD2 were labeled with [^{35}S]methionine during either exponential phase of growth (a–c) or histidine starvation (d–f). proteins were extracted as described in section 2, separated by isoelectric focusing (IEF) in the first dimension and polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) in the second dimension, and finally autoradiographed. The asterisk indicates the origin of migration. The arrowheads show some examples of proteins which are accompanied by satellite spots specifically in strains TA1001 and PD2 under starvation. They point, in each case, to the location of the qualitatively normal protein. (a and d) Strain TA997; (b and e) strain TA1001; (c and f) strain PD2.

pseudouridylate synthetase, has a role, unrelated to the modification of tRNAs, in the control of translational accuracy. Another possibility could be that unmodified charged tRNAs, such as the tRNA^{Gln} isoacceptors during histidine starvation, are unable to induce a conformational state in some constituent of the proteosynthetic machinery required for accurate translation. Finally, one cannot exclude the possible involvement of certain effectors whose synthesis and function are not

directly dependent on the *hisT* gene expression, but merely concomitant with it. One of these regulatory molecules could be the guanosine tetraphosphate ppGpp which is generally assumed to increase the fidelity of translation [13,14]. In this respect, we have previously noticed that the *hisT* mutant TA1001 of *S. typhimurium* used here, which carries the *relA*$^+$ genotype, exhibits an unusual behavior with regard to ppGpp accumulation under amino acid starvation [15]. In addition, our present data show that the *hisT* mutant PD2 frequently mistranslates the genetic program in a relaxed background. Further studies are now needed to determine the plausibility of these various hypotheses.

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