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*A SPECIFIC MODIFICATION OF LEUCYL-sRNA OF ESCHERICHIA COLI AFTER PHAGE T2 INFECTION**

BY NOBORU SUEOKA AND TAMIKO KANO-SUEOKA

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

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The metabolic transition of *Escherichia coli* infected with bacteriophage T4 from the early to the late phases[1] presents a unique problem in differentiation of gene expression. The mechanism for this major shift in the pattern of protein synthesis has not been understood. We have investigated the possible involvement of sRNA in the *E. coli* B–phage T2 system by comparison of aminoacyl-sRNA with and without phage infection, using the methylated albumin-kieselguhr (MAK) column fractionation technique.[2] Among sRNA's for 17 amino acids examined, only leucyl-sRNA showed an appreciable difference. Moreover, only some of the components of host leucyl-sRNA seemed to be modified by the phage infection. The injection of phage DNA and protein synthesis after the infection were found to be necessary for this sRNA modification.

Materials and Methods.—Strains: *E. coli* B, bacteriophage T2, and yeast (a strain of baker's yeast) were used.

Condition of phage T2 infection: *E. coli* B was grown overnight at 37°C in 50 ml nutrient broth medium (8 gm Difco nutrient broth, 5 gm NaCl in 1 liter H_2O, pH 7.0), and diluted 20 times with the same medium. When the cell concentration reached 6×10^8/ml, the phage was added, yielding a multiplicity of 10. Three minutes after the infection, aliquots were taken to assay infective centers and noninfected bacteria. In all of the experiments reported in this paper, noninfected bacteria were less than 2% of the infected bacteria, in most cases less than 0.5%. After an appropriate period of infection, the infected bacteria were chilled as quickly as possible in a beaker containing ice bags, which was immersed in ice water with salt. Then the infected bacteria were collected by centrifugation. sRNA and the aminoacyl-sRNA synthetase were prepared by the same method as for normal bacteria, and will be described below.

T2 infection in the presence of chloramphenicol: Chloramphenicol (50 μg/ml) was added 1 min before T2 infection. The multiplicity of the infection was 10. The total volume of the culture was 600 ml, and aliquots of 200 ml were taken at 5 min and 8 min after the infection to obtain sRNA. To show recovery of the infection process, at the 8th min after infection, 200 ml of the culture was chilled, centrifuged, washed once with an equal volume of a cold medium, and resuspended in a warm medium without chloramphenicol. The culture was then shaken vigorously

at 37°C for 8 min and harvested to purify sRNA. A portion of the culture was kept for an additional 30 min to assay the burst size, which was about 40.

Adsorption of T2 ghosts to E. coli: The ghosts of phage T2 were prepared as follows. The phage was suspended in a 3.8 *M* NaCl solution for 10 min, then diluted instantly 15 times with a diluting solution (NH_4Cl, 2 gm; NaCl, 5 gm; KCl, 0.37 gm; $MgCl_2 \cdot 6H_2O$, 0.01 gm; and Na_2SO_4, 0.026 gm, in 1 liter of tris-HCl 0.1 *M*, pH 7.3). After osmotic shock, the infectivity dropped to 1.0% of the control. The ghosts were adsorbed to *E. coli* at a multiplicity of 5.

sRNA and aminoacyl-sRNA synthetase: The sRNA was prepared by the phenol procedure described by von Ehrenstein and Lipmann.[3] The enzyme fraction was prepared free from RNA by passing the crude extract through a DEAE cellulose column.[4] sRNA's were charged with either H^3-leucine (5000 mc/mM, New England Nuclear Corp., Boston, Mass.) or C^{14}-leucine (240 mc/mM, New England Nuclear Corp.) and fractionated on methylated albumin columns according to the method previously reported,[4] except that the reaction mixture was incubated for 15 min at 37°C, and 0.05 *M* sodium phosphate buffer, pH 6.3, was used for the elution. The differential counting of C^{14} and H^3 was done in a Packard Tricarb liquid scintillation counter.

Results.—sRNA from *E. coli* infected with bacteriophage T2 for 8 min was charged with either C^{14}-amino acids or H^3-amino acids by an enzyme fraction obtained from T2 8-min-infected cells. These were fractionated on MAK columns as described in *Materials and Methods.* The profiles for 17 amino acids other than asparagine, glutamine, and cysteine were examined and compared with ones from noninfected *E. coli* cells. In only one amino acid was there a clear difference found in the profile between sRNA isolated from the infected cells and from the noninfected cells. As shown in Figure 1, the leucyl-sRNA of *E. coli* shows two major

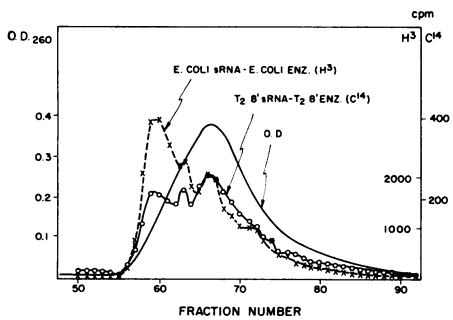

FIG. 1.—Elution profile of leucyl-sRNA of T2-infected *E. coli*. *E. coli* cells were infected with T2 at the multiplicity of 10. At the 8th min the culture was chilled quickly and the cells were harvested. sRNA was purified by the phenol method, and the crude extract was used to charge C^{14}-leucine onto the sRNA. The T2-infected sRNA was chromatographed together with *E. coli* sRNA charged with H^3-leucine on a MAK column. The starting buffer was 0.2 *M* NaCl and the final concentration was 1.1 *M*.

peaks in MAK column chromatography, leu I and leu II, leu I being the majority component.[2] The RNA from cells infected with T2 for 8 min shows that leu I decreases to less than half of the noninfected case.

In order to examine which was modified by phage infection, leucyl-sRNA or leucyl-sRNA synthetase, leucyl-sRNA from T2 infected cells was charged with enzyme from the noninfected cells and compared with the same sRNA charged with enzyme from 8-min-infected cells. As Figure 2*C* shows, the RNA charged with the enzyme from noninfected cells gives the same profile as the one charged with enzyme from 8-min-infected cells. This indicates that the change must be in the sRNA and not in the synthetase. In the following experiments, the enzyme from noninfected cells was used exclusively.

The time course of the change of the profiles of leucyl-sRNA was followed by preparing sRNA from the cells 3 min, 5 min, and 8 min after the infection. The elution profile of leucyl-sRNA from 3-min-infected cells showed a new component in front of the leu I peak not seen in the 8-min RNA (Fig. 2*A*). Five minutes after

infection, the new component increased, while the leu I peak decreased considerably (Fig. 2*B*). In leucyl-sRNA from 8-min-infected cells the new front component disappeared (Fig. 2*C*).

Two possibilities exist as to the origin of the new component: (1) appearance of a completely new sRNA, (2) partial degradation of the leu I peak. To examine these two possibilities, T2-infected sRNA was charged with radioactive leucine by yeast enzyme which was known to charge leucine only to the leu I peak of *E. coli* sRNA.[4] The results are presented in Figure 3. In the 3-min and the 5-min-infected sRNA,

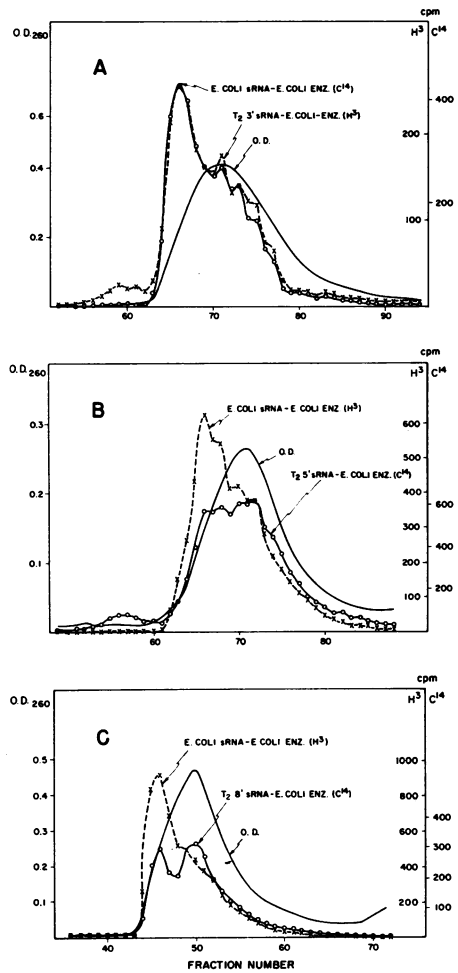

FIG. 2.—Leucyl-sRNA at different times after T2 infection. The sRNA was taken from the cells infected for 3 min, 5 min, and 8 min and charged by *E. coli* enzyme free from RNA. The profiles of the leucyl-sRNA were compared with that of *E. coli* by eluting together on a MAK column. (*A*) T2 3-min sRNA; (*B*) T2 5-min sRNA; (*C*) T2 8-min sRNA.

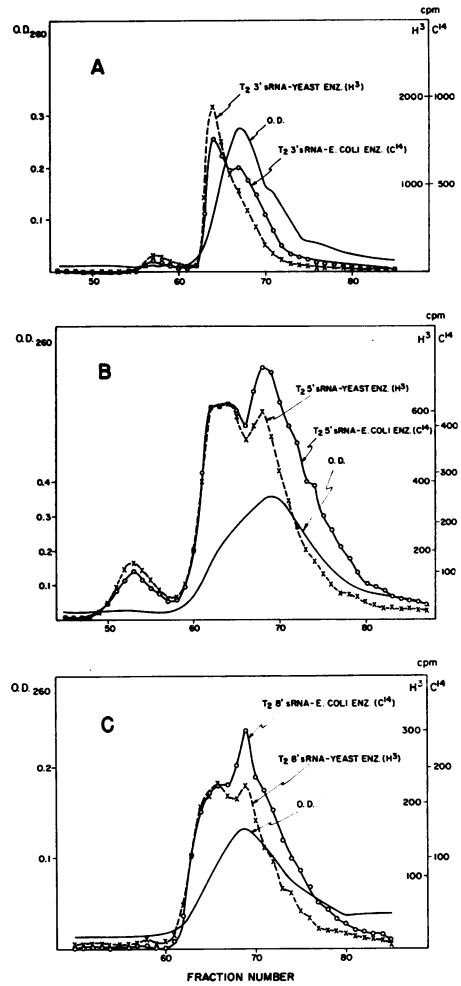

FIG. 3.—Leucyl-sRNA of T2 infected cells charged by yeast enzyme. Enzyme extract of yeast was freed from RNA by a DEAE cellulose column and used to charge radioactive leucine to T2-infected sRNA. The elution profile of the leucyl-sRNA was compared with that of T2-infected sRNA charged by *E. coli* enzyme. As in Fig. 2, sRNA's from the cells infected for (*A*) 3 min, (*B*) 5 min, and (*C*) 8 min were examined.

in addition to the leu I peak, the new front component was also charged. This indicates that the new peak is most likely the derivative of leu I. In the 5-min and 8-min sRNA some leucine was also incorporated into the leu II region when charged with yeast enzyme. Therefore, this also seems to be the modified product of leu I. The new component in the leu II region did not exist at the 3rd min, appeared by the 5th min, and increased and remained at the 8th min. One likely picture, therefore, is as follows. Some component of leu I is modified first to the front leu peak, and then further modified to the one in the leu II region. In this connection, since leu I is further fractionated into three components (Ia, Ib, and Ic) by a countercurrent distribution technique,[5] it is likely that only one of the three components of leu I is modified by the T2 infection. A quantitative estimation of the new components indicates that the total leucine acceptor activity seems to remain constant.

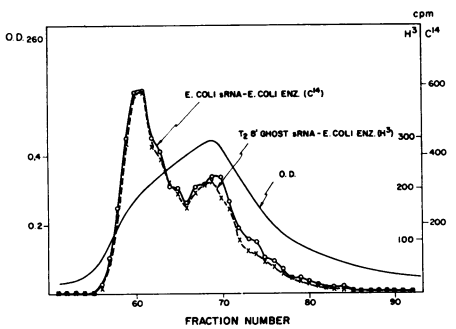

FIG. 4.—Leucyl-sRNA from the cells adsorbed with ghost. The ghosts of phage T2 were prepared as described in *Materials and Methods*. sRNA was prepared from the cells adsorbed with the ghosts for 8 min at a multiplicity of 5, charged wth H^3-leucine by *E. coli* enzyme, and eluted from a MAK column along with *E. coli* sRNA labeled with C^{14}-leucine. The survival cells after 3-min adsorption were 4%.

The necessity of injection of phage DNA for this modification was examined by attaching osmotically shocked phage (ghost) to the cells. sRNA was prepared from the cells 8 min after the adsorption. Optical density of the culture decreased to 90 per cent of the original value after the 8-min adsorption. Leucyl-sRNA did not show any modification in this process (Fig. 4). This implies that the phage DNA is necessary for the modification of the normal sRNA.

If the modification occurred by the action of a newly formed modifying enzyme, protein synthesis should be necessary after the phage infection. To examine this possibility, protein synthesis was prevented by the addition of chloramphenicol (50 μg/ml) 1 min before the phage infection. Details of the experimental procedure are described in *Materials and Methods*. Both the leucyl-sRNA's taken 5 min and 8 min after the infection were similar in profile to that of noninfected *E. coli* (Fig. 5*A* and *B*). Consequently, the new protein synthesis seemed necessary for the modification of leucyl-sRNA to occur. After the cells were infected with T2 for 8 min in the presence of chloramphenicol, the cells were washed and resuspended in the fresh medium free from chloramphenicol and the culture was shaken at 37°C for an additional 8 min. Leucyl-sRNA from this culture indicated a tendency to recover from the chloramphenicol effect; namely, the leu I peak decreased to some extent compared with the normal case (Fig. 5*C*). The small extent of the modification of leucyl-sRNA after washing may be due to the fact that the process of phage growth is slower than in a normal condition. Protein synthesis resumed slowly at the 5th min after resuspending the cells in a fresh medium.

Discussion.—The role of sRNA as the adaptor between messenger RNA and protein[6] has been reasonably well demonstrated.[7] Thus, the degeneracy of the code is reflected in a multicomponent of sRNA for each amino acid.[8] The translation of

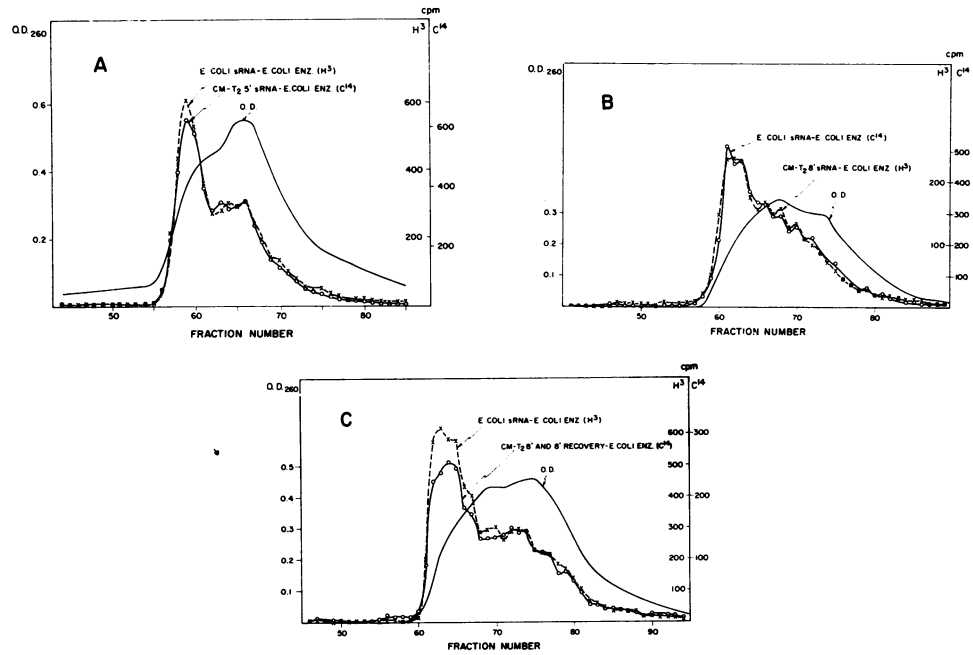

FIG. 5.—Leucyl-sRNA from *E. coli* infected with T2 in the presence of chloramphenicol. *E. coli* was infected with T2 at a multiplicity of 10 in the presence of 50 μg/ml chloramphenicol (*CM* in the fig.). The noninfected bacteria 3 min after infection were 0.008%. sRNA was charged by *E. coli* enzyme. (*A*) T2 5-min sRNA in the presence of chloramphenicol charged with C^{14}-leucine, (*B*) T2 8-min sRNA in the presence of chloramphenicol charged with H^3-leucine, (*C*) C^{14}-leucyl sRNA from the cells infected with T2 for 8 min in the presence of chloramphenicol and incubated an additional 8 min after removal of chloramphenicol. All profiles were directly compared with the normal *E. coli* leucyl-sRNA.

messenger RNA, therefore, should be affected if a change in the adaptor is introduced. Upon the establishment of a new fractionation technique of aminoacyl-sRNA on a methylated albumin-kieselguhr (MAK) column,[2] we started a search for a possible example of the modification of sRNA in relation to the regulation of protein synthesis. Our operating hypothesis is as follows. If the codon recognition of a particular adaptor out of a set of degenerate adaptors for an amino acid is changed by a structural modification, the mRNA of the genes which accomodate the codon corresponding to the modified adaptor should not be translated properly, while mRNA of the other genes which do not accommodate the codon should be translated normally. This means that by modifying a specific sRNA molecule, the function of some of the genes which are transcribed can be shut off and the rest of the genes kept functional at the translation level. This, if true, should constitute one of the major principles in differentiation and may be called the *adaptor modification* hypothesis. It is noted that this hypothesis may be applied to cases where a drastic change in the metabolic pattern is observed rather than to cases involving a change in a small number of enzymes. The *modulation* hypothesis proposed by Ames and Hartman[9] based on their experimental results on the histidine pathway is primarily to account for an alteration in translation of mRNA by a genetic change in a corresponding cistron and not in a change of pre-existing sRNA, while the present hypothesis is to account for a change of the spectrum of functioning genes during

development by modifying sRNA without provoking mutation of genes. The two hypotheses, therefore, are not mutually exclusive but deal with two different aspects of the regulation. Recently, a speculative extension of the modulation hypothesis has been made by Stent[10] with the purpose of explaining the repressor-induction (or derepression) scheme of protein synthesis proposed by Jacob and Monod.[11] The relation of Stent's scheme to our hypothesis and the finding reported in this paper should become clearer in the future.

The functional significance of the leucyl-sRNA modification is presently not clear. If the present hypothesis has any reality, the modification may not only be responsible for the transition from early to late phases of phage protein synthesis, but affect the translation of preformed host mRNA. The chemical nature of the modification is also unknown. However, the modification cannot be the removal of CCA terminal of sRNA,[12] because the modified sRNA apparently can accept leucine. Methylation[13] is an interesting possibility, although the high degree of specificity (the fact that only some leucyl-sRNA is modified) for the modification makes it less likely.

Summary.—A specific structural modification of leucyl-sRNA of *E. coli* B upon phage T2 infection was discovered by methylated albumin column fractionation of aminoacyl-sRNA. Among 17 amino acids examined (all except asparagine, glutamine, and cysteine), only one or two components of leucyl-sRNA showed a clear alteration after the phage infection. The modification started at the 3rd minute after infection and was completed by the 8th minute. The injection of phage DNA and protein synthesis were required for the modification. The possible implication of the modification on the transition of protein synthesis from the early to the late phase has been discussed.

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