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CONSERVATION OF SPECIFICITY BETWEEN AMINO ACID ACCEPTOR RNA AND AMINO ACYL-sRNA SYNTHETASE

BY TETSUO YAMANE AND NOBORU SUEOKA

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

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Several amino acids can be attached to the soluble RNA (sRNA) of one organism by amino acyl-sRNA synthetases of other organisms.[1-4] The nature of the cross reactions can be studied further by chromatographically identifying the amino acyl-sRNA formed under such conditions. Some results obtained by methylated albumin column fractionation have been reported.[5] Further studies with column fractionation of amino acyl-sRNA, formed by the interspecific combination of sRNA and the synthetase, have resulted in one of the following situations: (*a*) no cross reaction was observed; (*b*) the same profile was secured as that of normal amino acyl-sRNA; (*c*) only some component or components of normal amino acid acceptor RNA were charged; (*d*) an entirely different profile was noted. The first three are commonly observed. When cross reactions are observed, normal components of sRNA for the particular amino acid are charged with the amino acid. The last situation occurs in only one case so far examined where yeast leucyl-sRNA is formed by an *E. coli* synthetase; the lcueyl-sRNA formed has an entirely different profile from those of normal yeast and *E. coli* leucyl-sRNA's. However, the significance of this exception is not clear, since the leucyl-sRNA obtained constitutes only one per cent of the normal yeast leucyl-sRNA. These results indicate that the specificity between sRNA and the activating enzyme for each amino acid is strikingly conserved among different organisms. This conservative feature is even more remarkable when the adaptor hypothesis for the role of sRNA in protein synthesis is considered.

Materials and Methods.—Bacteria: The following strains were used: *Escherichia coli* B, *Pseudomonas aeruginosa* (American Type Culture Collection #10197), *Bacillus subtilis* (W23), *Micrococcus lysodeikticus* (ATCC #4698), *Aerobacter aerogenes* (ATCC #9624), *Salmonella typhimurium* (LT-2). Yeast: a strain of baker's yeast.

Preparation of sRNA: The sRNA was prepared by the phenol procedure described by von Ehrenstein and Lipmann.[6] To remove any attached amino acids, the sRNA preparation was incubated in 0.5 *M* Tris HCl, pH 8.8, for 45 min at 37°C. This suspension was then brought to 1 *M* of NaCl concentration, and sRNA precipitated by the addition of 2 vol ethanol, dissolved in H_2O, dialyzed against cold distilled water overnight, and lyophilized.

Preparation of enzyme extract: Essentially the method of Takanami and Okamoto[7] and Zubay[8] was used. Bacteria were grown at 37°C with constant shaking in enriched broth and yeast in a medium containing glucose (4%), peptone (0.5%), yeast extract (0.25%), ammonium sulfate (0.2%), KH_2PO_4 (0.1%), $MgSO_4$ (0.025%), and $CaCl_2$ (0.025%), pH adjusted to 4.8–5.0 (HCl)

The cells were harvested in the logarithmic phase (A_{660}: 0.3–0.5) and ground with 3 times their wet weight of alumina (levigated alumina from Norton Abrasives, Worcester, Mass.). To the crude extract from a one-liter culture, 3 ml of Tris-magnesium buffer (0.01 *M* Tris-HCl buffer, pH 7.3 plus 0.01 *M* $MgCl_2$) were added, and the mixture was centrifuged at 105,000 × *g* for 3 hr at 0°C. The upper 2/3 of the supernatant was dialyzed against 500 ml Tris-magnesium buffer plus 0.006 *M* mercaptoethanol at 4°C for 3 hr, changing the outside buffer every half hour.

Preparation of enzyme fraction free from RNA: 105,000 × *g* supernatant was applied to a 1.2 cm × 5 cm column of DEAE cellulose equilibrated with phosphate buffer (0.02 *M*, pH 7.7) containing 0.006 *M* mercaptoethanol. The charged column was rinsed with 50 ml of the phosphate buffer, and the enzyme eluted with 0.02 *M* potassium phosphate buffer containing 0.35 *M* NaCl (pH 7.7). Fractions were collected in 2 ml portions, and tubes with high absorbancies were combined and used for esterification of amino acids to sRNA.

Preparation of amino acyl-sRNA: C^{14}-labeled amino acyl-sRNA was prepared basically according to Berg *et al.*[3] The reaction mixture contained the following compounds totaling 0.5 ml: 50 μmoles of Tris buffer (pH 7.3); 0.5 μmole of ATP, 5 μmoles of $MgCl_2$; 0–1 mg of sRNA; 2 μmoles of reduced glutathione; 5 μmoles of KCl, 0.01–0.02 ml of enzyme extract, an appropriate amount of C^{14}-amino acid plus 19 remaining nonradioactive amino acids (1 μmole of each); and, unless otherwise stated, the reaction mixture was incubated at 37°C for 25 min. Amino acyl-sRNA was isolated by the phenol procedure of Gierer and Schramm.[9] Radioactive amino acids used were: from Calbiochem, Los Angeles, Calif., L-methionine 4.5 μc/μmole (specific activity); from New England Nuclear Corp., Boston, Mass., L-leucine C^{14} 143.4 μc/μmole, L-lysine C^{14} 144 μc/μmole, L-phenylalanine C^{14} 369 μc/μmole, L-proline C^{14} 115 μc/μmole, L-methionine H^3 14.1 μc/μmole, DL-leucine H^3 5400 μc/μmole, DL-phenylalanine H^3 30 μc/μmole, DL-proline H^3 5000 μc/μmole.

Incorporation experiments: Essentially the method of Nirenberg and Matthaei[10] was followed for the preparation of *E. coli* extracts (preincubated, DNAase-treated, S-30 fractions) and for reaction mixtures used for determining C^{14}-amino acid incorporation into protein. The reaction mixture (0.5 ml) contained the following components: 50 μmoles Tris pH 7.8; 5 μmoles magnesium acetate; 25 μmoles KCl; 3 μmoles mercaptoethanol; 25 μmoles *M* PEP; 10 μg of PEP-kinase (Calbiochem); 0.15 μmoles GTP; 10 μg of polynucleotide; 0.05 mg of C^{12}-amino acyl-sRNA omitting leucine; 0.02 ml of the incubated S-30 fraction, and C^{14}-leucyl-sRNA. The poly UC (base ratio 2.1:1) and UG (2.8:1) were kindly donated by Dr. M. W. Nirenberg, and poly U by Dr. J. Fresco.

Methylated albumin column: The preparation of this column was simplified by pouring 30 ml of MAK (mixture of kieselguhr and methylated albumin in phosphate buffer) directly into the column (31 mm i.d.) as the first layer, and a suspension of 1 gm kieselguhr in 0.2 *M* saline buffer on top as a protective layer. The MAK was prepared by suspending 6 gm of kieselguhr in 30 ml of 0.05 *M* sodium phosphate buffer, pH 6.7, followed by boiling and cooling the suspension. 1.5 ml of 1% methylated albumin solution in H_2O was stirred in slowly.[11]

Results.—Our approach to the study of the nature of interspecific cross reaction between sRNA and the amino acyl-sRNA synthetase is qualitative as well as quantitative; namely, the nature of the cross reaction is examined from elution patterns of amino acyl-sRNA on a methylated albumin column. Soluble RNA's were isolated by the phenol method, and extracts containing amino acyl-sRNA synthetases free from RNA were prepared by chromatography on a DEAE-cellulose column. Extracts were checked for enzyme activity using homologous sRNA. Contamination of homologous sRNA in the extract was proved negligible: P^{32}-labeled sRNA mixed with crude extract was totally retained on the DEAE cellulose column after elution with an 0.35 *M* saline buffer solution. Contamination was also examined from time to time by incubating the fractionated enzyme extract with the radioactive amino acid in question, adding homologous nonradioactive sRNA as a carrier, and immediately isolating the sRNA by the phenol method. Fractionation of such RNA gave no radioactive peak.

In Table 1 the esterifying of a radioactive amino acid to sRNA by the heterologous enzyme is expressed in percentage against that by the homologous enzyme.

Phenylalanine: As shown in Figure 1, the perfect matching of the two yeast phenylalanyl-sRNA preparations, one using a homologous (yeast) enzyme and the other a heterologous (*E. coli*, or *Ps. aeruginosa*) enzyme, indicates that amino acyl-sRNA synthetases from the three sources have the same specificity. A similar effect is observed with *E. coli* sRNA and the enzymes from *Ae. aerogenes*, *B. subtilis*, and *M. lysodeikticus*. In the *E. coli*-yeast combination, yeast amino acyl-sRNA synthetase fails to esterify phenylalanine to *E. coli* sRNA, although the *E. coli* enzyme is able to attach phenylalanine onto yeast sRNA. The failure of *E. coli* sRNA to accept phenylalanine by yeast enzyme could be attributed to a special RNAase present in the yeast enzyme extract, which partially degrades *E. coli* sRNA, or, more specifically, the acceptor end of the sRNA. This possibility was examined by checking the phenylalanine acceptor activity using *E. coli* amino acyl-sRNA synthetase. No difference was observed, either qualitatively or quantitatively, between *E. coli* sRNA treated or untreated with yeast enzyme extract (Fig. 2), indicating that the action of RNAase, which may exist in yeast extract, is unlikely.

TABLE 1

EXTENT OF AMINO ACID ATTACHMENT TO sRNA BY HETEROLOGOUS AMINO ACYL-sRNA SYNTHETASES

sRNA	Synthetase	Methionine	Phenyl-alanine	Leucine	Lysine	Proline
E. coli	*E. coli*	100%	100%	100%	100%	100%
	Yeast	37	2	74	59	0.9*
	S. typhimurium (LT-2)		91*	95*		
	B. subtilis (W23)		99*			
	Ae. aerogenes		99*			
	M. lysodeikticus		58*			
Yeast	Yeast	100%	100%	100%	100%	100%
	E. coli	60	12	0.8	70	3*
S. typhimurium (LT-2)	*S. typhimurium* (LT-2)		100%	100%		
	E. coli		81*	104*		

Relative amino acid acceptor activity of *E. coli*, yeast, and *S. typhimurium* sRNA's when assayed with enzyme extracts from various sources. Assay conditions are as described under *Materials and Methods*. The values given are the average of several experiments, normalized to the value obtained for each sRNA with homologous enzyme. The figures with asterisks are based on one set of experiments.

Proline: Amino acyl-sRNA synthetase from *E. coli* could not utilize yeast sRNA, and vice versa (Fig. 3).

Leucine: Previously we reported that the leucyl-sRNA of yeast formed by the enzyme of *E. coli*, although small in amount, is different in profile from the normal yeast leucyl-sRNA,[12] and that leucyl-sRNA of *E. coli* formed by the yeast enzyme has a profile covering the front part of the normal *E. coli* leucyl-sRNA.[5] Figures 4*A* and 5*A* show that the amount of leucyl yeast-sRNA formed by the *E. coli* enzyme is about one per cent of that formed by yeast enzyme. As shown in Figure 5, the amount of leucyl-sRNA thus formed is proportional to the amount of yeast sRNA in the reaction mixture. The reciprocal combination, *E. coli* sRNA and yeast enzyme, formed leucyl-sRNA in about two thirds of the normal *E. coli* quantity. The elution profile from the methylated albumin column is shown in Figures 6*A* and 6*B*. The cause of the "strange" leucyl-sRNA profile observed in yeast-

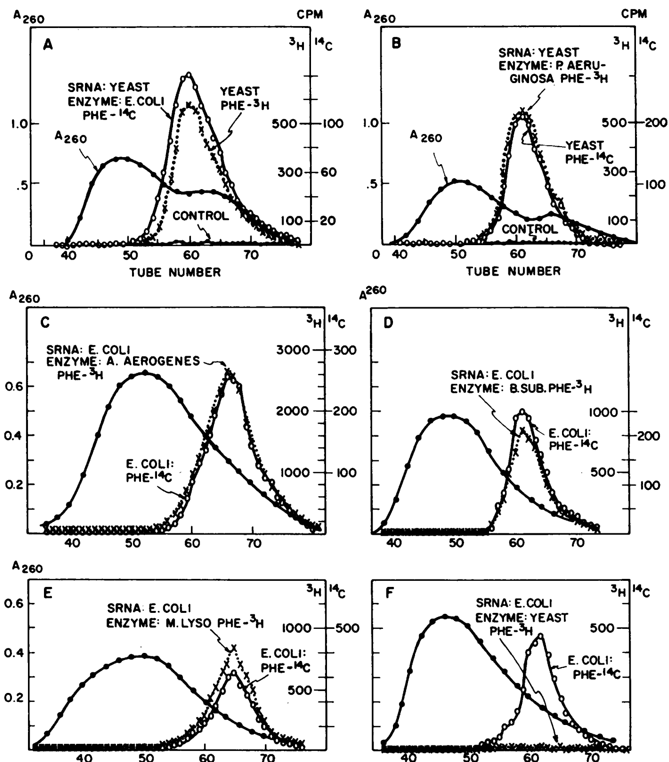

FIG. 1.—Interspecific exchange of sRNA and the amino acyl-sRNA synthetase for the formation of phenylalanyl-sRNA. Comparison of the chromatographic profiles of normal yeast C^{14}-phenylalanyl-sRNA and yeast H^3-phenylalanyl-sRNA obtained, using *E. coli* enzyme extract (*A*), and *Ps. aeruginosa* (*B*), normal *E. coli* C^{14}-phenylalanyl-sRNA with *E. coli* H^3-phenylalanyl-sRNA formed with enzyme extract from *Ae. aerogenes* (*C*), *B. subtilis* (*D*), *M. lysodeikticus* (*E*), and yeast (*F*). For comparison, normal *E. coli* or yeast phenylalanyl-sRNA was mixed with the product of heterologous enzymes and chromatographed on a simplified methylated albumin column. The differential counting of C^{14} and H^3 was done in a Packard Tri-Carb liquid scintillation counter. For control, no sRNA was added during the incubation period, but after chilling and addition of cold phenol the same amount of sRNA was added as in the heterologous combination. The sRNA was isolated and chromatographed as in the text.

sRNA and the *E. coli* enzyme combination may be attributed to one of the following possibilities: (1) radioactive leucine preparations being contaminated by another amino acid; (2) yeast sRNA being modified by some enzymes present in the *E. coli* enzyme extract; (3) the "strange" peak being a minor component of normal yeast leucyl-sRNA; (4) leucine being esterified to a yeast sRNA which normally does not bind leucine. The first possibility is not likely, because removal of 19 other nonradioactive amino acids from the reaction mixture did not change the

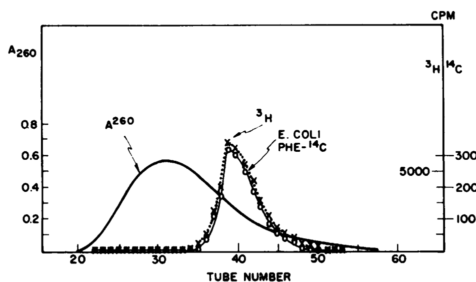

FIG. 2.—*E. coli* sRNA after treatment with yeast enzyme extract as described in *Methods*, tested for phenylalanine (H^3) acceptor activity using homologous (*E. coli*) enzyme. *E. coli* C^{14}-phenylalanyl-sRNA added for comparison.

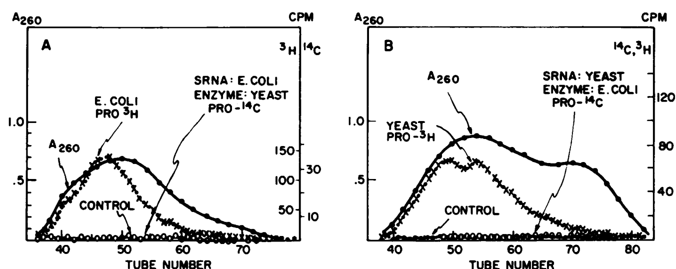

FIG. 3.—Comparison of chromatographic profiles of (*A*) *E. coli* H^3-prolyl-sRNA and *E. coli* sRNA treated with yeast enzyme extract for the attachment of C^{14}-proline; (*B*) yeast H^3-prolyl-sRNA and yeast sRNA treated with *E. coli* amino acyl-sRNA synthetase for the binding of C^{14}-proline.

result (100 times in excess of radioactive leucine), while the addition of nonradioactive leucine eliminated the peak. As to the second possibility, Nishimura and Novelli[13] reported that the treatment of *E. coli* leucyl-sRNA with *B. subtilis* RNAase causes a shift in the methylated albumin-kieselguhr chromatographic profile. To test this possibility, yeast sRNA charged with C^{14}-leucine by the *E. coli* enzyme was discharged by pH 8.8 incubation and recharged with C^{14}-leucine, using the homologous enzyme. The elution profile of the resulting sRNA is shown with the normal yeast H^3-leucyl-sRNA (Fig. 7). The perfect fitting of the two leucyl-sRNA's disproved the second possibility. In this case, since the reaction mixture for treating yeast sRNA with the *E. coli* enzyme contained methionine and ATP, the possible effect of methylation on the profile should have been noted in the final elution pattern. The absence of modification of the profile tended to exclude also the methylation of yeast sRNA by the *E. coli* enzyme extract. The third possibility was examined by refractionating the "strange" peak to see whether a component of the normal leucyl-sRNA corresponded to the "strange" peak. Owing to the small amount of the "strange" component, the result was not conclusive. The last possibility also cannot be excluded. In spite of various experiments attempting to identify the "strange" peak as a normal component among the yeast amino acid acceptor RNA, the results have not been clear. The transfer of leucine from the "strange" peak to poly UC and poly UG are shown in Figure 8*A*.

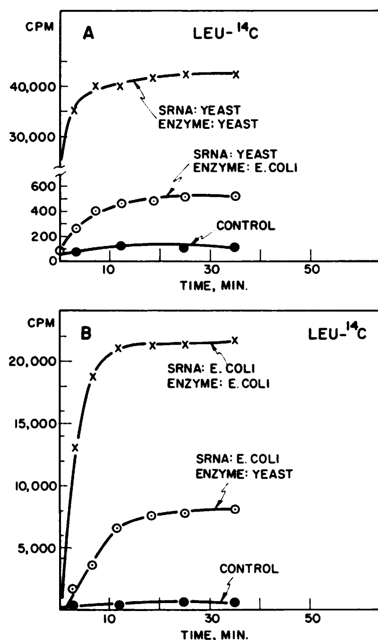

FIG. 4.—Kinetics of leucyl-sRNA formation. The conditions used were those described for the usual assay of amino acyl-sRNA formation. For control, no sRNA was added during the incubation period, but after chilling and addition of cold 10% TCA, 0.1 mg of sRNA was added.

Methionine: The interspecific exchange of sRNA and enzyme between *E. coli* and yeast shows that only a part of the methionine acceptor RNA of each organism can be charged with heterologous enzyme (Fig. 9*A*). The result on the methionyl-sRNA formed by *E. coli* sRNA and yeast enzyme confirms the original finding of Berg *et al.*[3] and shows that a similar situation exists for the reciprocal

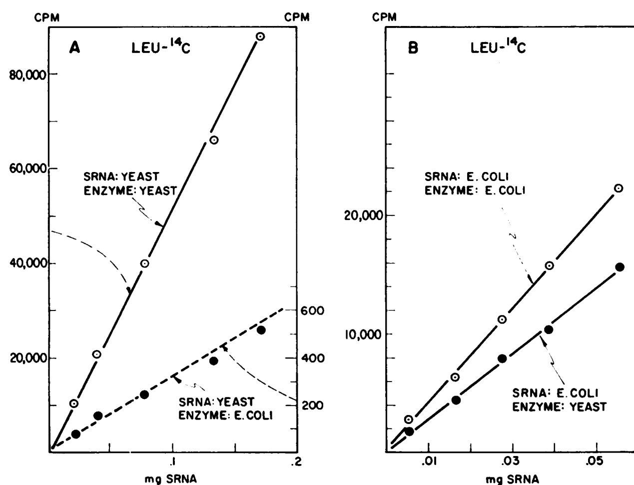

FIG. 5.—Attachment of C^{14}-leucine to yeast sRNA (*A*) and *E. coli* sRNA (*B*) by enzymes from either organism. In reaction mixtures, the same amount of C^{14}-leucine was added to the homologous and heterologous combination. Incubation was made at 37°C for 30 min, and the reaction was stopped by adding 10% TCA (final concentration).

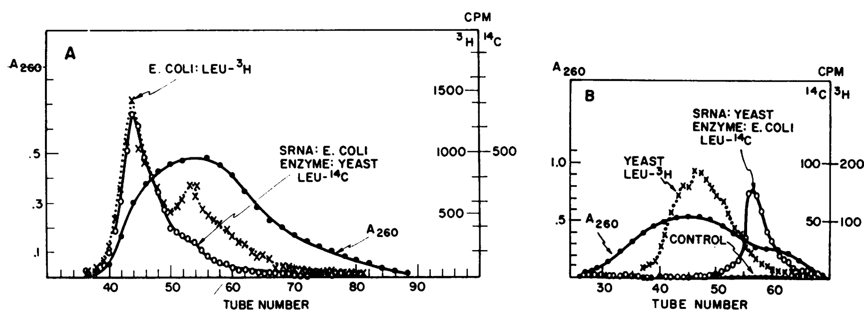

FIG. 6.—Comparison of the chromatographic profiles of (*A*) *E. coli* H^3-leucyl-sRNA and *E. coli* C^{14}-leucyl-sRNA obtained by using yeast amino acyl-sRNA synthetase, and (*B*) yeast H^3-leucyl-sRNA and yeast C^{14}-leucyl-sRNA formed by *E. coli* enzyme extract.

combination (Fig. 9*B*). In our previous report,[12] we mentioned that the methionyl-sRNA formation between *E. coli* and yeast showed a "strange" peak as in the case of leucine. However, the result was proved wrong since the enzyme extract had been contaminated with homologous sRNA.

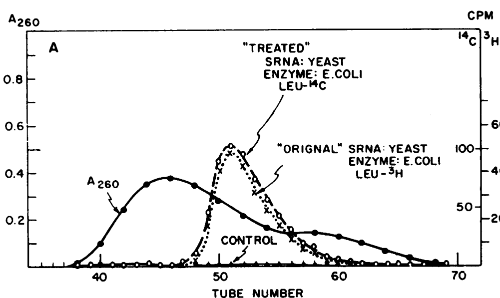

FIG. 7.—Effect of *E. coli* extract on the profile of the "strange" yeast leucyl-sRNA formed by *E. coli* extract. "Treated": yeast sRNA, after treatment with *E. coli* enzyme extract and H^3-leucine, was discharged and recharged with C^{14}-leucine esterified using *E. coli* enzyme. "Original": yeast sRNA charged with H^3-leucine by *E. coli* enzyme.

Lysine: Between *E. coli* and yeast, the profile of the lysyl-sRNA formed by heterologous enzymes showed exactly the same profiles as those of the normal lysyl-sRNA (Fig. 10).

Discussion.—The chromatographic analysis of the amino acyl-sRNA produced by interspecific cross reactions between sRNA and the amino acyl-sRNA synthetase indicated that all or some of the normal components of amino acyl-

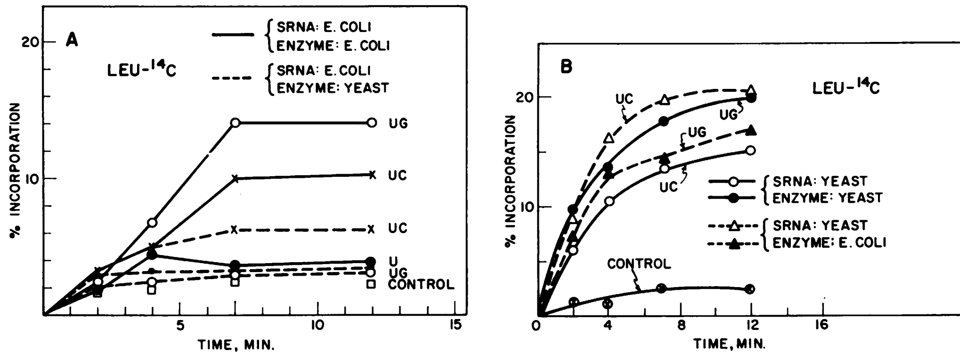

FIG. 8.—(*A*) Polymer-dependent incorporation of *E. coli* C^{14}-leucyl-sRNA obtained by using homologous and yeast enzymes. See text for conditions. Each point represents: —O—O—, —x—x—, —●—●—, 0.020 mg of sRNA with 7,728 cpm; ---O---O---, ---x---x---, ---●---●---, 0.035 mg sRNA containing 6,541 cpm. Control contained all components, except polymer. (*B*) Polymer-dependent incorporation of yeast C^{14}-leucyl-sRNA obtained by using homologous and *E. coli* enzymes. Each point represents: —O—O—, —●—●— 0.014 mg of sRNA, 7,224 cpm; ---△---△---, ---▲---▲--- 0.98 of sRNA, 3,274 cpm.

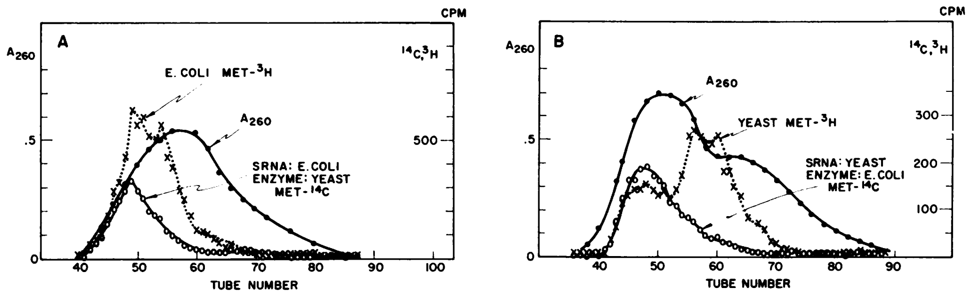

FIG. 9.—Comparison of the chromatographic profiles of (*A*) *E. coli* H^3-methionyl-sRNA and *E. coli* C^{14}-methionyl-sRNA obtained by using yeast enzyme extract; (*B*) yeast H^3-methionyl-sRNA and yeast C^{14}-methionyl-sRNA formed by *E. coli* enzyme.

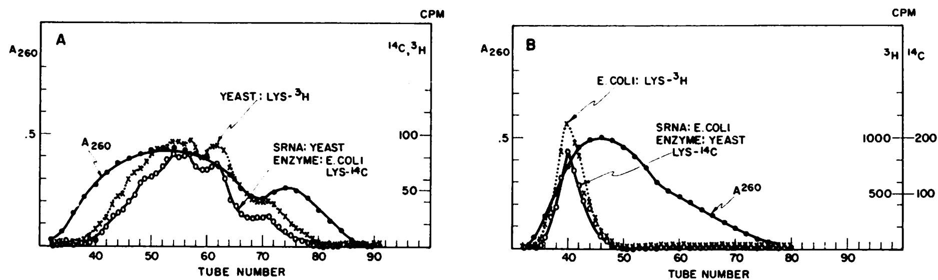

FIG. 10.—Comparison of the chromatographic profiles of (*A*) normal yeast H^3-lysyl-sRNA and yeast C^{14}-lysyl-sRNA obtained using *E. coli* enzyme; (*B*) normal *E. coli* H^3-lysyl-sRNA and *E. coli* C^{14}-lysyl-sRNA formed by yeast enzyme.

sRNA were charged with the particular amino acid. One possible exception has been found in the formation of yeast leucyl-sRNA by the *E. coli* enzyme. However, owing to the relatively small amount, the nature and significance of this exception is not clear.

The conserved specificity is quite surprising; in the adaptor hypothesis the

conservation of specificity in the level of amino acyl-sRNA and the amino acyl-sRNA synthetase is not required, even if the code is universal.[6, 14–16] In view of the unusual behavior on the column of yeast leucyl-sRNA formed by the *E. coli* enzyme, the cross reaction should be examined more extensively before a general conclusion is given. However, the fortuitous fitting between the enzyme-recognizing site of the acceptor RNA of one organism and a part of the activating enzyme of the other organism, irrespective of the amino acid specificity,[12] turned out to be unlikely among the organisms used in the present work.

Bennett, Goldstein, and Lipmann[17] report that although a part of the *E. coli* leucine acceptor RNA is compatible with the yeast enzyme, the *E. coli* enzyme did not charge the yeast leucine acceptor RNA. This does not contradict our result on the same case, since only a small amount of yeast leucyl-sRNA (about 1% of the normal case) is formed by the *E. coli* enzyme.

The first indication of the conserved feature of the specificity was reported by Berg *et al.*[3] who showed that the isolated methionyl-sRNA synthetase from yeast could attach methionine to a part (40%) of the *E. coli* methionine acceptor RNA. Recently, Bennett *et al.*[17] analyzed, in greater detail, yeast leucyl-sRNA formed by *E. coli* enzyme. Their result shows that only a component of the *E. coli* leucine acceptor RNA (peak I of Weisblum *et al.*[18]) accepts leucine by yeast enzyme. Our data for the same combinations are consistent with their results.

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