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ON THE SPECIES SPECIFICITY OF ACCEPTOR RNA AND ATTACHMENT ENZYMES

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One of the steps in protein biosynthesis appears to be the attachment of each amino acid to a specific acceptor (SRNA) molecule. According to the adaptor hypothesis, each SRNA molecule would then fit to a specific complementary base sequence on a linear RNA template, specifying the sequence of amino acids in the resultant protein.[1, 2] An adaptor molecule thus could have two specificities: one recognizing the correct amino acid and activating enzyme; the other, the proper position on the template. The correctness of the amino-acid sequence therefore would depend upon the precision and constancy of the adaptors. However, the structures of the enzymes and adaptors are presumably under the genetic control of the organism and might be subject to heritable modifications. It is therefore conceivable that one or both ends of an adaptor might change sufficiently to cause occasional errors and, in the long run, an alteration of the genetic code might evolve. This notion, prompted by genetic observations[3] which suggested that mutation of a bacterium might modify its translation of genetic information, lead to the present comparison of the specificities of the acceptor RNA and activating enzymes of different organisms.

Several differences in specificity have been reported previously. Berg *et al.*[4] demonstrated that SRNA from *Escherichia coli* contains two distinguishable acceptors for methionine. An enzyme prepared from yeast could attach methionine to one of these, while the enzyme from *E. coli* could attach to both. Webster found, in pig liver, a difference between the nuclear and cytoplasmic attachment enzymes for alanine. Rendi and Ochoa[6] noted that, for leucine, the enzymes in

yeast and in *E. coli* could attach only to their homologous SRNA. Furthermore, in the case of leucine, rat liver enzyme and SRNA were interchangeable with those from *E. coli*.

The observations presented below show that whether the enzymes and/or acceptors from two organisms are interchangeable depends upon not only the organisms in question but also the particular amino acid.

Materials and Methods.—Preparation of SRNA: SRNA from *E. coli* was prepared by the method of Ofengand *et al.*[7] (but omitting the fractionation on ECTEOLA) and dissolved in ammonium formate buffer (0.6 *M*, pH 4.7). The solution had an optical density at 260 mμ of 123 measured by dilution in 0.1 *M* NaCl at pH 9. Yeast SRNA was prepared from bakers' yeast (Anheuser Busch Co.) according to the method of Holley *et al.*[8] and dissolved in formate buffer. The solution had an optical density at 260 mμ of 139 measured by dilution in 0.1 *M* NaCl at pH 9.

Preparation of enzyme extracts: Extracts containing amino acyl-RNA synthetase activity were obtained by the method of Bergmann *et al.*[9] Suspensions of the cells or tissue (*E. coli*, yeast, or rabbit liver) in Tris buffer, 0.025 *M*, pH 8.0, were stirred with fine glass beads in a Waring blendor, and diluted after blending to a final concentration of 1 gm wet weight of cells per 8 ml buffer. The homogenates were centrifuged (20,000 *g* for 1 hr) and the supernatant solutions were dialyzed overnight against 40 volumes of buffer (0.01 *M* Tris, pH 8.0). The preparations were stored frozen at −20°C and thawed prior to use. *C^{14} amino acids* were obtained from the California Corporation for Biochemical Research. The specific activities were: L-arginine, 5.0 μc/μM; L-tyrosine, 1.2 μc/μM; DL-lysine, 0.94 μc/μM.

Assay of amino acid acceptor activity of SRNA: The procedure used was that of Berg *et al.*[4] The reaction mixture contained the following ingredients, in a total volume of 0.5 ml: 50 μ moles of sodium cacodylate buffer, pH 7.0; 0.5 μ mole of ATP; 5 μ moles of $MgCl_2$; 0 to 4 units of SRNA; 0.2 μc of C^{14}-L-arginine, or 0.2 μc of C^{14}-L-tyrosine, or 0.4 μc of C^{14}-DL-lysine; 100 μg of crystalline beef serum albumin; 2μ moles of reduced glutathione; 5μ moles of KCl; and 0.06 ml of dialyzed enzyme extract. One unit of SRNA represents 0.06 ml of the SRNA solution in formate buffer. Assuming an optical density at 260 mμ of about 24 for a solution of 1 mg/ml, this corresponds to approximately 0.3 mg of RNA. Unless otherwise stated, the reaction mixture was incubated at 30°C for 25 minutes. The mixture was then chilled in an ice bath, and 3 ml of cold salt-ethanol (0.5 *M* NaCl, 67% ethanol) added to precipitate the SRNA. SRNA of the same kind was then added as carrier, making the total amount of SRNA constant (4 units) in all tubes. After 5 minutes, the tubes were centrifuged (6,000 *g*, 5 minutes) in the cold. The pellets were resuspended in cold salt-ethanol and the centrifugation cycle repeated three times more. Finally, the samples were dissolved in 1 ml. of 1.5 *M* NH_4OH, dried on planchets, and counted with a thin window gas flow counter.

Some of the observed counts may be expected due to SRNA present in the enzyme preparation itself and some due to coprecipitation of labelled amino acid with SRNA and protein after the addition of salt-ethanol. The sum of these was measured in a control. To this tube, no SRNA was added during the incubation period, but, after chilling and addition of salt-ethanol, four units of SRNA were added. As can be seen in the figures, the number of counts in such control tubes was relatively insignificant.

Results.—A. *Comparison of E. coli and yeast for three amino acids:* (1) Arginine: Curve A in Figure 1 shows the acceptor activity for C^{14}-arginine of SRNA from *E. coli*, assayed with an enzyme extract from the same organism. The conditions were such that the SRNA was maximally labelled, as shown by kinetic experiments, for homologous combinations of SRNA and enzyme extract. Note that the amount of C^{14}-arginine attached is proportional to the amount of SRNA added. Furthermore, as shown by the solid circle, incubation for twice the length of time, with twice as much enzyme, and twice as much C^{14}-arginine does not increase the number of counts significantly. Thus, the amount of SRNA is the limiting factor, and the slope of the curve is a measure of specific acceptor activity.

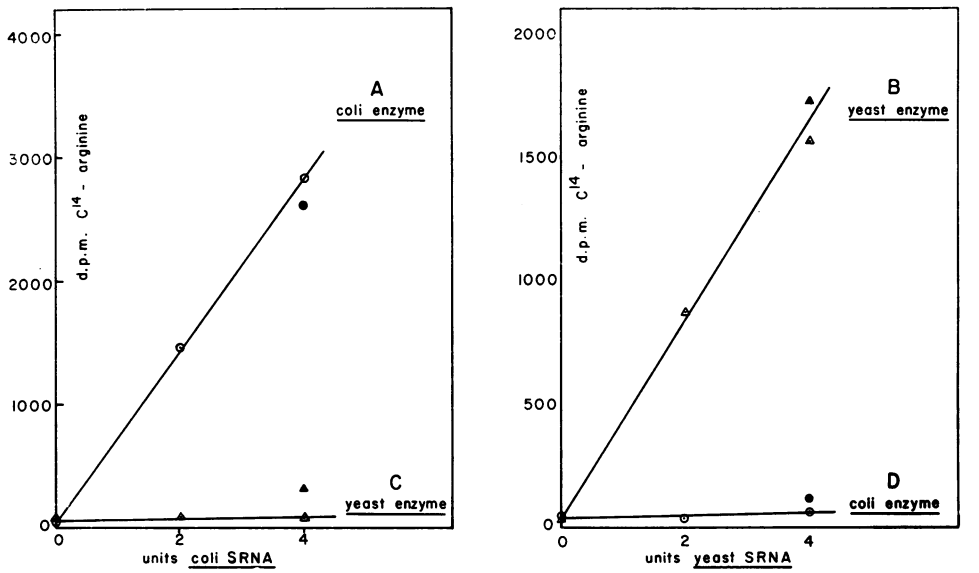

FIG. 1.—Attachment of C^{14}-arginine to *E. coli* SRNA and to yeast SRNA using enzyme preparations from either organism. The conditions are designed so that the SRNA, when treated with the homologous enzyme, becomes saturated with C^{14}-amino acid. The solid points represent samples incubated for twice the regular time, with twice the regular concentration of enzyme and twice the regular concentration of C^{14}-amino acid.

Curve B shows the activity of yeast SRNA assayed with yeast enzyme. In contrast, curves C and D for the heterologous mixtures show very little response indeed. (In the latter cases, slightly more attachment does occur in the controls with more enzyme, amino acid, and time of incubation, suggesting that the inability of the enzyme extract to catalyze attachment is not absolute.) This experiment shows that the arginine acceptors in *E. coli* and yeast are different and also that the attachment enzymes are different.

(2) Tyrosine: The same experiment for tyrosine is shown in Figure 2. Again, the strong homology requirement shows that both the acceptors and the enzymes for tyrosine are different in *E. coli* and yeast. Therefore, for at least three amino acids, arginine, tyrosine, and leucine, the enzymes and acceptors in yeast and in *E. coli* are almost completely non-interchangeable.

(3) Lysine: Turning now to the amino acid lysine, a different pattern is obtained. As shown in Figure 3, curves A and C, either the *E. coli* or the yeast enzyme extract is effective in attaching lysine to *E. coli* SRNA. However, it would be premature to conclude from this that the two enzymes are identical in their specificity. Testing the same enzyme extracts with yeast SRNA, curves B and D, shows them to be different. Furthermore, the fact that the yeast SRNA discriminates between the two enzymes, while the *E. coli* SRNA does not, shows that the SRNA's are not identical in the two organisms. The curves suggest that yeast SRNA may contain at least two different acceptors for lysine.

(4) Other amino acids: Preliminary studies with other amino acids indicate that various patterns are possible, with various degrees of interchangeability of the enzymes and/or the SRNA's. However, complete interchangeability for both

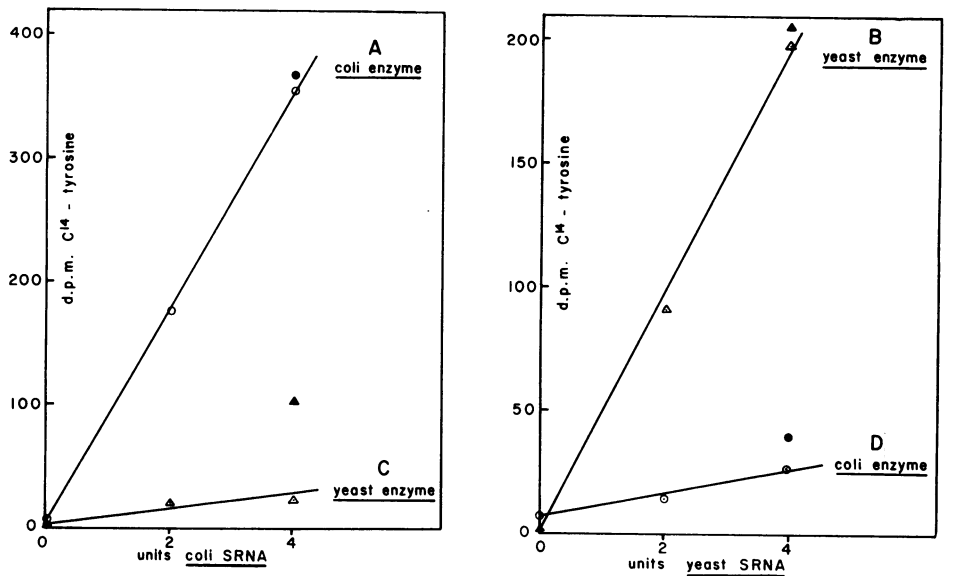

FIG. 2.—Attachment of C^{14}-tyrosine. Conditions same as for Figure 1.

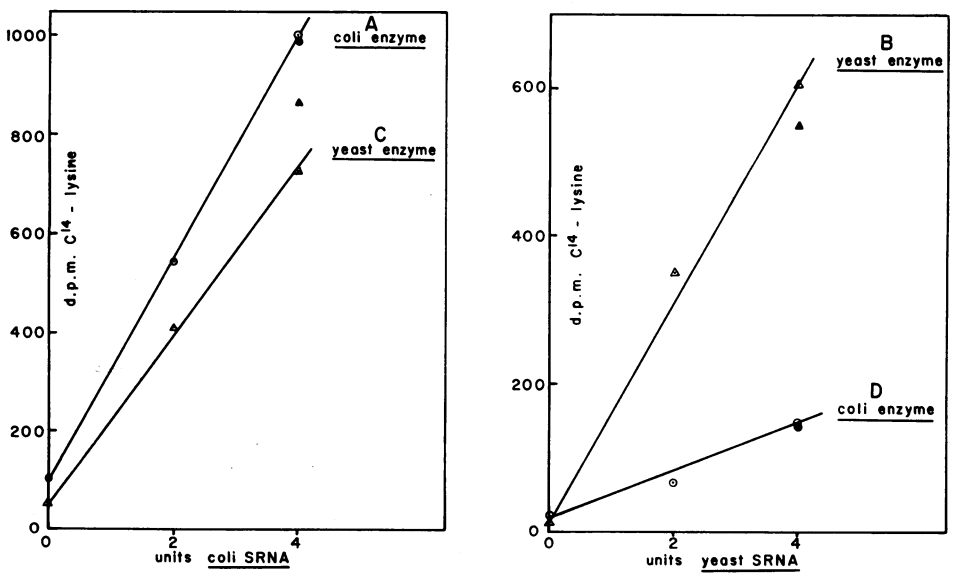

FIG. 3.—Attachment of C^{14}-lysine. Conditions same as for Figure 1.

the enzymes and the SRNA's of *E. coli* and yeast for a given amino acid is certainly not the rule.

B. *Rabbit enzyme:* The acceptor activity of SRNA from *E. coli* and yeast has also been assayed for two amino acids using an enzyme extract from rabbit liver. As shown in Table 1, rabbit liver enzyme extract attaches arginine to *E. coli* SRNA as efficiently as does the *E. coli* enzyme extract, but is ineffective with yeast SRNA. For tyrosine, however (Table 2), the situation is reversed; the rabbit enzyme specificity resembles more closely that of yeast.

TABLE 1

ATTACHMENT OF C^{14}-ARGININE

Enzyme extract	*E. coli* SRNA	Yeast SRNA
E. coli	1.00	0.02
Yeast	0.01	1.00
Rabbit	1.18	0.02

Relative arginine acceptor activities of *E. coli* SRNA and yeast SRNA when assayed with enzyme extracts from *E. coli*, yeast, or rabbit liver. The values given are the slopes of the curves, measured as in Figure 1, normalized to the value obtained for each SRNA with homologous enzyme.

TABLE 2

ATTACHMENT OF C^{14}-TYROSINE

Enzyme extract	*E. coli* SRNA	Yeast SRNA
E. coli	1.00	0.10
Yeast	0.05	1.00
Rabbit	0.01	1.12

Relative tyrosine acceptor activities of *E. coli* SRNA and yeast SRNA when assayed with enzyme extracts from *E. coli*, yeast, or rabbit liver. The values given are the slopes of the curves, measured as in Figure 1, normalized to the value obtained for each SRNA with homologous enzyme.

If a mixture of the coli and rabbit enzyme extracts is used with *coli* SRNA, the amount of arginine attached is no greater than with rabbit enzyme alone. This implies that both enzymes attach arginine to the same acceptor. The same applies to the attachment of tyrosine to yeast SRNA by a mixture of yeast and rabbit enzyme extracts.

Discussion.—The present results may bear only on one part of the SRNA molecule—that determining which amino acid is attached. It is conceivable that, for a given amino acid, the portion of its SRNA molecule that determines the specific attachment to the template is identical in all organisms. In that case the genetic code would remain universal. However, since the specificity of interaction of the SRNA molecule with the enzyme catalyzing attachment of a particular amino acid is subject to wide species variations, it is not difficult to imagine that the SRNA molecule could change sufficiently to accept a different amino acid. If this has happened in nature (and if the adaptor hypothesis is correct) the genetic code might well not be universal.

A direct demonstration of this could come from an experiment in which a given amino acid, attached to the SRNA from one organism, were to become incorporated in place of another amino acid when added to the protein synthetic machinery of a second organism. This did not occur in one such experiment performed by von Ehrenstein and Lipmann[10] where amino acids attached to *E. coli* SRNA (using *E. coli* enzymes), were efficiently incorporated into rabbit hemoglobin, and the leucine, which was labelled with C^{14}, apparently went into its normal positions in the protein. It may be desirable to extend such experiments to other amino acids with SRNA from species which show as little as possible activation with the amino-acyl-RNA synthetases from rabbit. To establish the universality of the code by this means would be difficult, whereas one instance could suffice to contradict it.

In the experiments described here, crude enzyme extracts were used. This was preferable in order to avoid the possibility of discarding any of the attachment enzymes. However, the question might be raised whether some role could be attributed to the enzyme-catalyzed repair mechanism which regenerates the required terminal sequence (CCA) at the acceptor ends of partially degraded SRNA

molecules. In this connection, it should be noted that addition of cytidine triphosphate (1 micromole) to the reaction mixtures did not increase the amount of arginine attached to either coli SRNA or yeast SRNA, with either homologous or heterologous enzyme extracts. Furthermore, since the repair enzyme (in coli) does not appear to be sufficiently specific to distinguish even between the acceptors for different amino acids,[11] it seems most unlikely to be responsible for the effects observed. In any case, specificity in the repair reaction would be an equally valid demonstration that the SRNA chains specific for a single amino acid are manifestly variable in different organisms.

While the species differences observed are quite striking, it is equally impressive that certain homologies should exist, as for a rabbit and a bacterium or for a rabbit and a yeast. This suggests that the number of possible variations in an SRNA acceptor for a given amino acid is severely restricted. The data, while still scanty, show sufficient variety to suggest that any generalization is precarious.

Summary.—The specificities of acceptor SRNA and attachment enzymes in *Escherichia coli* and yeast are compared. Striking differences are found, but the pattern depends upon the particular amino acid in question. Enzyme from rabbit liver resembles *E. coli* enzyme for one amino acid (arginine), but for another amino acid (tyrosine) it resembles yeast enzyme. The implications of these facts for the problem of the universality of the genetic code are discussed.

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