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RNA CODEWORDS AND PROTEIN SYNTHESIS, III. ON THE NUCLEOTIDE SEQUENCE OF A CYSTEINE AND A LEUCINE RNA CODEWORD

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Previous studies utilizing randomly ordered synthetic polynucleotides to direct amino acid incorporation into protein in *E. coli* extracts indicated that RNA codewords corresponding to valine, leucine, and cysteine contain the bases (UUG).[1-4] The activity of chemically defined trinucleotides in stimulating the binding of a specific C^{14}-aminoacyl-sRNA to ribosomes, prior to peptide bond formation,[5] provided a means of investigating base sequence of RNA codewords and showed that the sequence of a valine RNA codeword is GpUpU.[6]

Although the template activity of poly UG in inducing the binding of C^{14}-Val-sRNA to ribosomes was found to be high, poly UG had little effect upon the binding of C^{14}-Leu-sRNA to ribosomes. These results differed markedly from the high template activity of poly UG in directing both leucine and valine incorporation into protein.[6] We have investigated this paradox further and have found that a small fraction of C^{14}-Leu-sRNA binds to ribosomes in response to poly UG. This technique has been employed to estimate the relative proportion of a C^{14}-aminoacyl-sRNA preparation which recognizes a poly- or trinucleotide. Experiments will be described which suggest that the nucleotide sequence of an RNA codeword corresponding to a Leu-sRNA component of fraction II is UpUpG, and the nucleotide sequence of a cysteine RNA codeword is UpGpU.

Materials and Methods.—Aminoacyl-sRNA preparation: Uniformly labeled C^{14}-L-leucine, C^{14}-L-phenylalanine, and C^{14}-L-valine with specific radioactivities of 160, 282, and 205 μcuries/μmole, respectively, were obtained from New England Nuclear Corp. S^{35}-cystine of specific radioactivity 39 μcuries/μmole was obtained from the Amersham Radiochemical Centre and was reduced to S^{35}-cysteine by the addition of β-mercaptoethanol. *E. coli* W 3100 sRNA, prepared by the method of Zubay,[7] was acylated with C^{14}-leucine, C^{14}-phenylalanine, C^{14}-valine, and S^{35}-cysteine by modifications of methods described previously.[8] Deacylated *E. coli* B sRNA used in the sRNA exchange experiments was obtained from General Biochemicals, Inc.

A partially purified cysteine activating enzyme was used to synthesize S^{35}-Cys-sRNA. The enzyme was purified by adjusting *E. coli* W 3100 100,000 $\times$ *g* supernatant solution to pH 5.0 with acetic acid, removing the precipitate by centrifugation, and treating the supernatant solution with streptomycin. The precipitate was removed by centrifugation, and solid $(NH_4)_2\ SO_4$ was added to the supernatant solution (to 50% saturation). The precipitate was collected by centrifugation and was dissolved in 0.01 *M* Tris, pH 7.2; 0.014 *M* magnesium acetate; 0.005 *M* β-mercaptoethanol; and 0.05 *M* KCl. After acylating the sRNA with S^{35}-cysteine, the reaction mixture was deproteinized by shaking with phenol saturated with H_2O as described previously.[8] The aqueous layer was passed through a column of Sephadex G-25, and the S^{35}-Cys-sRNA was then lyophilized.

E. coli B C^{14}-Leu-sRNA fractions IA, IB, and II, separated by countercurrent distribution, were the generous gift of Drs. Bernard Weisblum and Gunther von Ehrenstein. Leu-sRNA fractions IA, IB, and II were well separated from one another and comprised approximately 29, 55, and 15% of the leucine acceptor activity, respectively. C^{14}-Leu-sRNA fractions IA and IB respond to poly UC during protein synthesis and correspond to Leu-sRNA fraction I described previously by Weisblum, Benzer, and Holley.[9] C^{14}-Leu-sRNA fraction II contains two Leu-sRNA components, IIA and IIB, recognizing poly U and poly UG, respectively, in the amino acid incorporating system (equivalent to Leu-sRNA fraction II of Weisblum *et al.*[9]).

Poly- and oligonucleotides: Poly UG and UC (designation, D123 and S-253, respectively) were prepared and characterized as described previously.[2, 10] The base ratio of poly UG was 0.74/0.26; of poly UC, 0.49/0.51. The preparation and characterization of the oligonucleotides used in this study have been described previously.[6]

Assay: Each 50-μl reaction mixture contained 0.10 *M* Tris acetate, pH 7.2; 0.05 *M* KCl; and 2.210 A^{260} units of *E. coli* W 3100 ribosomes (3 $\times$ washed). Reaction mixtures with S^{35}-Cys-sRNA, C^{14}-Phe-sRNA, or C^{14}-Val-sRNA contained 0.02 *M* magnesium acetate; those with C^{14}-Leu-sRNA contained 0.03 *M* magnesium acetate, unless otherwise specified. Conditions of incubation, oligo- or polynucleotide, and C^{14}-aminoacyl-sRNA added are indicated in the table legends. C^{14}-aminoacyl-sRNA bound to ribosomes was determined as described previously.[5]

Results.—Poly UG preparations with different proportions of U and G have been shown to direct approximately equal amounts of valine, leucine, and cysteine into protein in cell-free *E. coli* extracts.[3, 4] The effect of poly UG and other polynucleotides upon the binding to ribosomes of S^{35}-Cys-sRNA, C^{14}-Leu-sRNA fractions II, IA, and IB, separated by countercurrent distribution, and unfractionated C^{14}-

TABLE 1

POLYNUCLEOTIDE-AMINOACYL-sRNA SPECIFICITY

Radioactive aminoacyl-sRNA present	Polynucleotide Added: Poly UG	Poly UC	Poly U	Poly A	None
	$\mu\mu$Moles Radioactive Aminoacyl-sRNA Bound to Ribosomes				
C^{14}-Leu-sRNA, frac. II	2.28	1.12	1.36	0.66	0.76
C^{14}-Leu-sRNA, frac. IB	0.48	0.90	0.28	0.38	0.40
C^{14}-Leu-sRNA, frac. IA	0.24	0.58	0.28	0.28	0.30
C^{14}-Leu-sRNA, unfrac.	0.67	1.10	0.70	—	0.70
S^{35}-Cys-sRNA	0.84	—	0.37	—	0.29

Where indicated, 7.4 $\mu\mu$moles of S^{35}-cysteine attached to 0.15 A^{260} units sRNA; 21 $\mu\mu$moles C^{14}-leucine attached to 0.15 A^{260} units unfractionated sRNA; 4.9 $\mu\mu$moles C^{14}-leucine attached to 0.024 A^{260} units Leu-sRNA fraction II; 6.3 $\mu\mu$moles C^{14}-leucine attached to 0.048 A^{260} units Leu-sRNA fraction IB; and 6.4 $\mu\mu$moles C^{14}-leucine attached to 0.072 A^{260} units Leu-sRNA fraction IA were added to each reaction mixture. 20 mμmoles (as base residues) of poly UG, poly UC, poly U, or poly A were added to reaction mixtures as indicated. Incubation was at 24° for 10 min.

Leu-sRNA is shown in Table 1. The binding of S^{35}-Cys-sRNA to ribosomes was directed by poly UG.

The binding to ribosomes of unfractionated C^{14}-Leu-sRNA was stimulated by poly UC, but not by poly UG or poly U. In contrast, 45 per cent of the C^{14}-Leu-sRNA fraction II was induced to bind to ribosomes by poly UG. This sRNA fraction also responded, but to a lesser extent, to poly U and to poly UC. Since C^{14}-Leu-sRNA fraction II has been found to contain two sRNA components accepting leucine, one with high specificity for poly U, the other for poly UG, the template activity of poly UC may be due to uridylic acid residues only. The binding of C^{14}-Leu-sRNA fractions IA and IB to ribosomes was stimulated by poly UC, but not by poly U, poly UG, or poly A. These results demonstrate the specificity of each C^{14}-Leu-sRNA fraction for codeword recognition, and agree with the specificity obtained by measuring C^{14}-leucine incorporation into protein in *E. coli* extracts.[9, 11, 13]

In Figure 1 is shown the relation between C^{14}-Leu-sRNA concentration and binding to ribosomes induced by poly UC and poly UG. In Figure 1*A*, reaction mixtures were incubated for 10 min at 24°, and immediately thereafter were washed. In Figure 1*B*, after incubating reaction mixtures for 10 min at 24°, deacylated sRNA was added, and reaction mixtures were incubated for an additional 2 min so that release of C^{14}-Leu-sRNA from ribosomes (perhaps exchange with unbound deacylated sRNA) could occur. As shown in Figure 1*A*, relatively high background binding was observed in the absence of synthetic polynucleotides, as reported pre-

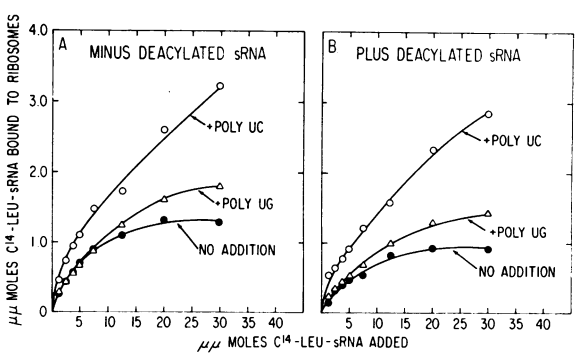

FIG. 1.—The effect of deacylated sRNA upon the exchange of poly UC (○) and poly UG (△) dependent, and background (●) binding of unfractionated C^{14}-Leu-sRNA to ribosomes. Where indicated, reaction mixtures contained poly UC, 20 mμmoles of base, and poly UG, 20 mμmoles of base. Incubation was at 24° for 10 min. *A* represents samples washed immediately after incubation; *B*, samples to which 3.5 A^{260} units of deacylated sRNA were added after 10 min of incubation; incubation was then continued for an additional 2 min prior to washing. Unfractionated C^{14}-Leu-sRNA (81 $\mu\mu$moles C^{14}-leucine attached to 1.0 A^{260} unit of sRNA) was added to each reaction mixture as indicated in the figure.

viously. Background C^{14}-Leu-sRNA binding was related to the concentration of C^{14}-Leu-sRNA within the range 0–12.5 $\mu\mu$moles of C^{14}-Leu-sRNA. Poly UG had little observable effect upon C^{14}-Leu-sRNA binding at low sRNA concentrations. However, binding was stimulated by poly UG at higher concentrations of sRNA. Poly UC stimulated binding at all C^{14}-Leu-sRNA concentrations tested; however, the stimulation was most apparent at higher sRNA concentrations.

Polynucleotide-induced binding of C^{14}-aminoacyl-sRNA has been found to be largely nonexchangeable with unbound sRNA.[5] The data of Figure 1*B* show that background and polynucleotide-induced C^{14}-Leu-sRNA binding were not freely exchangeable. Since the background binding was lower, the polynucleotide-induced binding of C^{14}-Leu-sRNA was slightly more apparent. In other experiments not presented here, deacylated sRNA was replaced with sRNA acylated with 20 C^{12}-amino acids. Similar results were obtained.

In Table 2 is shown the specificity of tri- and dinucleotides in stimulating the binding to ribosomes of S^{35}-Cys- and C^{14}-Leu-sRNA. The trinucleotide UpGpU stimulated the binding of S^{35}-Cys-sRNA, whereas UpUpG, GpUpU, and UpUpU, as well as the dinucleotides UpG, GpU, and UpU, were without effect. UpGpU had no effect upon the binding of C^{14}-Leu-sRNA or, as previously reported, upon the binding C^{14}-Val-, or C^{14}-Phe- sRNA.[6]

TABLE 2

OLIGONUCLEOTIDE-AMINOACYL-sRNA SPECIFICITY

	$\mu\mu$Moles C^{14}-Aminoacyl-sRNA Bound to Ribosomes				
		C^{14}-Leu-sRNA			
Addition	S^{35}-Cys-sRNA	Unfractionated	II	IB	IA
None	0.29	1.08	0.76	0.40	0.30
UpGpU	1.46	0.96	0.78	0.40	0.28
UpUpG	0.32	1.02	1.74	0.36	0.34
GpUpU	0.34	0.86	0.92	0.34	0.30
pUpUpU	—	0.87	0.80	0.38	0.26
UpUpU	0.32	—	—	—	—
UpG	0.21	0.92	0.92	—	—
GpU	0.34	0.80	0.86	—	—
UpU	0.26	0.88	0.88	—	—

Where indicated, S^{35}-Cys-sRNA, unfractionated C^{14}-Leu-sRNA, C^{14}-Leu-sRNA II, C^{14}-Leu-sRNA IA, and C^{14}-Leu-sRNA IB were added to reaction mixtures in the amounts specified in the legend of Table 1. Where indicated, 20 mμmoles (as base residues) of the trinucleotides UpGpU, UpUpG, GpUpU, pUpUpU, UpUpU, and 15 mμmoles (as base residues) of the dinucleotides UpG, GpU, and UpU were added to reaction mixtures. Incubation was at 24° for 10 min.

The addition of trinucleotides had no apparent effect upon the binding of unfractionated C^{14}-Leu-sRNA. However, the trinucleotide UpUpG stimulated the binding to ribosomes of purified C^{14}-Leu-sRNA fraction II, whereas UpGpU, GpUpU, pUpUpU and the dinucleotides UpG, GpU, and UpU were without effect. The binding of C^{14}-Leu-sRNA fractions IA or IB were not stimulated by any of the tri- or dinucleotides tested. These data indicate that UpUpG and UpGpU stimulate with specificity the binding to ribosomes of C^{14}-Leu-sRNA II and S^{35}-Cys-sRNA, respectively.

It is important to emphasize that of the three purified C^{14}-Leu-sRNA fractions, only fraction II recognized poly UG and UpUpG. Although poly U was found to induce the binding C^{14}-Leu-sRNA II to ribosomes, the trinucleotide pUpUpU had no binding activity for this sRNA fraction. We have previously observed this phenomenon in experiments with unfractionated C^{14}-Leu-sRNA.[5]

In Table 3, the effects of deacylated sRNA upon the release from ribosomes of C^{14}-aminoacyl-sRNA, induced to bind by trinucleotides and by polynucleotides, are compared. In experiment 1, reaction mixtures were incubated with poly UG or UpUpG to induce interactions between ribosomes and C^{14}-Leu-sRNA II. After binding to ribosomes had occurred, an excess of deacylated sRNA was added to one set of reactions and incubated for an additional 3 min to permit release of bound C^{14}-Leu-sRNA II.

TABLE 3

EFFECT OF DEACYLATED sRNA UPON STABILITY OF C^{14}-AMINOACYL-sRNA RIBOSOME COMPLEXES FORMED IN PRESENCE OF TRI- OR POLYNUCLEOTIDE TEMPLATES

Expt. no.	Addition	—μμMoles C^{14}-Aminoacyl-sRNA Bound to Ribosomes— Minus deacylated sRNA	Plus deacylated sRNA
1		C^{14}-Leu-sRNA II	
	None	0.64	0.52
	Poly UG	1.78	1.43
	UpUpG	1.42	1.37
2		C^{14}-Val-sRNA	
	None	0.57	0.27
	Poly UG	1.92	1.16
	GpUpU	1.43	1.19

Each reaction mixture contained the components noted under *Materials and Methods*, with the following exception: C^{14}-Val-sRNA reaction mixtures contained 0.03 *M* magnesium acetate. 6.44 μμmoles C^{14}-leucine attached to 0.032 A_{260} units Leu-sRNA fraction II, and 8.4 μμmoles C^{14}-valine attached to 0.11 A_{260} units sRNA were added to reaction mixtures where indicated. Ten mμmoles (as base residues) of poly UG, GpUpU, or UpUpG were added where indicated. Incubation was at 24° for 15 min. Where indicated, 3 A_{260} units of deacylated sRNA were added after 15 min incubation, and incubation was continued for an additional 3 min.

C^{14}-Leu-sRNA II induced to bind to ribosomes either by UpUpG or by poly UG was not readily exchangeable with unbound, deacylated sRNA. In experiment 2, the release of C^{14}-Val-sRNA, induced to bind to ribosomes by GpUpU or by poly UG, was determined. C^{14}-Val-sRNA release in the presence of deacylated sRNA was higher than the release of C^{14}-Leu-sRNA in experiment 1; however, little difference between the stability of the tri- compared to the polynucleotide-induced complex was observed.

Since the proportion of C^{14}-Leu-sRNA which responded to the RNA codeword UpUpG was small in comparison to the total C^{14}-Leu-sRNA, it was of interest to determine the relative proportions of unfractionated C^{14}-Leu-, C^{14}-Phe-, and C^{14}-Val-sRNA which could be induced to bind to ribosomes by appropriate codewords. In Figure 2*A* is shown the relation between C^{14}-Phe-sRNA concentration and binding of C^{14}-Phe-sRNA to ribosomes stimulated by poly U and by pUpUpU; in Figure 2*B*, C^{14}-Val-sRNA binding directed by poly UG; and in Figure 2*C*, unfractionated C^{14}-Leu-sRNA binding directed by poly UG and poly UC. Poly U directed the binding to ribosomes of 100 per cent of the C^{14}-Phe-sRNA in the reaction mixture at all C^{14}-Phe-sRNA concentrations used. Binding stimulated by pUpUpU was proportional to C^{14}-Phe-sRNA concentration within the range 0–1.24 μμmoles, and approximately 66 per cent of the available C^{14}-Phe-sRNA bound to ribosomes. Although it is clear that essentially all of the C^{14}-Phe-sRNA recognized poly U templates, it is not known whether the lower binding directed by pUpUpU is due to a less efficient assay or to two species of C^{14}-Phe-sRNA. As shown in Figure 2*B*, poly UG stimulated the binding of 61 per cent of the C^{14}-Val-sRNA present. In other experiments, approximately 80 per cent of the C^{14}-Val-sRNA present was bound. In contrast, the data of Figure 2*C* demonstrate that poly UG

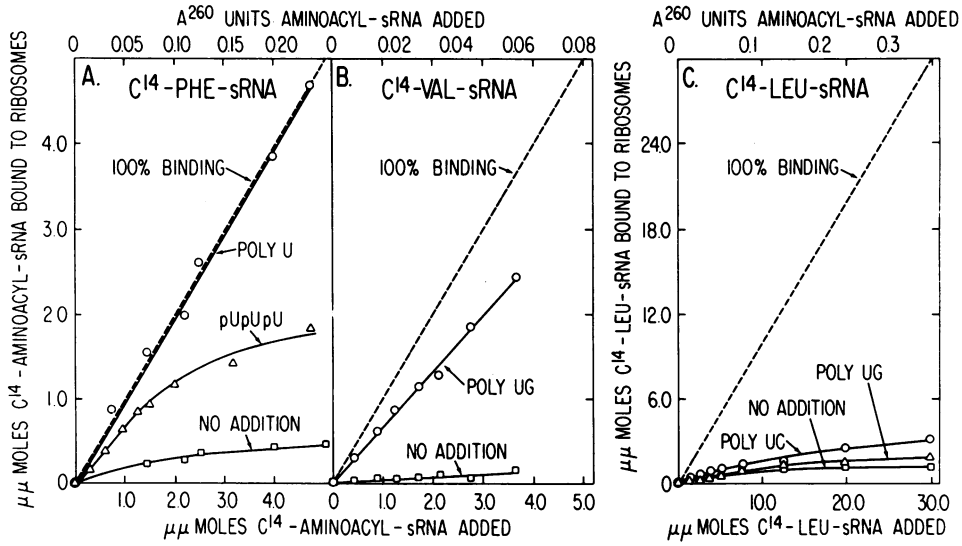

FIG. 2.—Comparison between the proportions of unfractionated C^{14}-Phe-, C^{14}-Val-, and C^{14}-Leu-sRNA binding to ribosomes in the presence of poly- and oligonucleotides. Reaction mixture components are described under *Materials and Methods*. C^{14}-aminoacyl-sRNA was added as indicated in each portion of the figure. The broken line (———) represents the theoretical binding of 100% of the C^{14}-aminoacyl-sRNA added. In *A*, the symbols represent the addition of: (O) poly U, 25 mμmoles of base residues; (△) pUpUpU, 25 mμmoles of base residues; (□) no addition. In *B*, the symbols represent the addition of: (O) poly UG, 15 mμmoles of base residues; (□) no addition. In *C*, symbols are: (O) poly UC, 20 mμmoles of base residues; (△) poly UG, 20 mμmoles of base residues; (□) no addition. Incubation was at 24° for 20 min in the case of C^{14}-Phe-sRNA; and for 10 min, in the case of C^{14}-Leu-sRNA.

and poly UC directed approximately 2 and 6 per cent, respectively, of the available C^{14}-Leu-sRNA to bind to ribosomes. The concentration of C^{14}-Leu-sRNA added to reactions was extended to 30 μμmoles, since the stimulation of binding by poly UG was more apparent at higher aminoacyl-sRNA concentrations. Similar results were obtained in experiments not presented here, with other conditions and times of incubation. These results suggest that less than 10 per cent of C^{14}-Leu-sRNA responds to RNA codewords which have thus far been assigned to leucine.

Discussion.—On the basis of experiments with synthetic polynucleotides directing amino acids into protein in *E. coli* extracts, RNA codewords containing the following bases, of unspecified base sequence, have been assigned to leucine: (UUC), (UCC), and (UUG).[1, 14, 26] An ambiguous codeword recognition between poly U and leucine also has been observed which is influenced markedly by a variety of environmental factors.[14–16] The RNA codeword (UUA) has been assigned to leucine, but has not been studied as extensively as the other assignments and may be due to uridylic acid residues only.

The heterogeneity of Leu-sRNA has been demonstrated in many different ways. Apgar and Holley[17] have separated by countercurrent distribution techniques five sRNA fractions from *E. coli* which accept leucine. Berg, Lagerkvist, and Dieckmann[18] have found at least two base sequences, GpCpApCpCpA and GpUpApCpCpA, at the 3′-terminus of *E. coli* Leu-sRNA. Differential response of Leu-sRNA fractions to leucine activating enzymes from different organisms have been reported.[12, 13, 19, 20, 27] In addition, preferential response of Leu-sRNA fractions to

poly UC, UG, and poly U during protein synthesis in *E. coli* extracts has been observed in different laboratories.[9, 11-13] Since leucine activating enzyme has been found by Yamane and Sueoka[21] to catalyze a transfer of the leucine moiety from one Leu-sRNA fraction to another, the observed specificities of sRNA fractions for different codewords may represent minimal values.

Poly UG preparations containing different proportions of U and G direct approximately equal amounts of C^{14}-valine and C^{14}-leucine into protein in *E. coli* extracts,[1-4] whereas poly UG directs the binding to ribosomes of considerably more C^{14}-Val- than C^{14}-Leu-sRNA.[6] It seems probable that sRNA functions *catalytically* during protein synthesis in crude extracts. In contrast, the use of poly- or trinucleotides to direct the binding of C^{14}-aminoacyl-sRNA to ribosomes may reflect other aspects of the codeword recognition process, i.e., the *proportion* of an aminoacyl-sRNA which attaches stably to ribosomes in response to a codeword.

Clearly, factors which affect either RNA codewords, or components related to codeword recognition, may affect the synthesis of a protein. The conditions of codeword recognition, such as pH, temperature, ionic environment, concentration of each sRNA and amino acid, acylated/deacylated sRNA ratio, the affinity with which a particular RNA codeword or sRNA binds to ribosomes, or the efficiency with which an activating enzyme acylates each sRNA species, and so forth, would be expected to influence this process. The binding assay affords opportunities to investigate many of these factors.

It seems probable that trinucleotides are recognized in correct phase. Certainly the high template specificity of each trinucleotide and the observations that oligonucleotides with three bases are active as templates, whereas dinucleotides are not, suggest a phased recognition of trinucleotides. However, these observations do not prove the phase of recognition, and other possibilities also deserve consideration.

In this study, the binding of C^{14}-Leu-sRNA fraction II to ribosomes was stimulated by UpUpG but not by UpGpU, GpUpU, pUpUpU, or dinucleotides. The binding of S^{35}-Cys-sRNA was stimulated by UpGpU but not by UpUpG, GpUpU, UpUpU, or dinucleotides. Assuming that trinucleotides are recognized in phase, the nucleotide sequence of an RNA codeword recognized by Leu-sRNA II would be UpUpG, and the sequence of a cysteine RNA codeword would be UpGpU. The remaining isomeric trinucleotide, GpUpU, was previously suggested to be an RNA codeword for valine.[6] Cramer, Küntzel, and Matthaei have suggested the same sequence for leucine.[29]

Utilizing the sequences indicated for cysteine, leucine, and valine codewords and

TABLE 4

PREDICTED NUCLEOTIDE SEQUENCES OF RNA CODEWORDS

Amino acid	Sequence
Leucine (sRNA fraction II)†	UpUpG
Cysteine†	UpGpU
Valine†	GpUpU
Isoleucine[23, 24]	ApUpU
	Ap (UA)
Tyrosine[28]	UpApU
Alanine[22]	GpCpC
	Gp (CA)
	Gp (CU)
Glutamic acid[22]	GpApA*
	GpApU
Glycine[22]	GpGpU
	GpGpC
	GpGpA*
Arginine[22]	ApGpA
	CpGpC
	(A)Gp(C)
Threonine[23, 24]	ApCpA
	(ACC)
Aspartic acid[22]	Gp(CA)

* These assignments, based upon limited data, are tentative.

† Derived experimentally.

Nucleotides within parentheses have not been arranged in sequence. Amino acid replacements used for these predictions were found in *E. coli* by Yanofsky and co-workers[22, 28] or were induced by HNO_2 in TMV by Wittman *et al.*[23] and also by Tsugita.[24]

amino acid replacement data, predictions concerning the base sequence of other RNA codewords can be made and are summarized in Table 4. Due to the degenerate nature of the code, many self-consistent schemes are possible. Only the most probable predictions have been presented.

It is important to emphasize that almost all C^{14}-Phe-sRNA formed a stable complex with ribosomes and poly U; 60–80 per cent of C^{14}-Val-sRNA, with poly UG; whereas only 2 and 6 per cent of unfractionated C^{14}-Leu-sRNA formed a stable complex with ribosomes in the presence of poly UG and poly UC, respectively. Although the affinity of one aminoacyl sRNA for a ribosome may differ from that of another sRNA, we have not observed a more rapid release of C^{14}-Leu-sRNA II from ribosomes, compared with the release of C^{14}-Val-sRNA. After separation by countercurrent distribution, Leu-sRNA fraction II (containing the A and B components) account for an estimated 15 per cent of the total leucine acceptor activity. It is not known whether the relative amount of each Leu-sRNA fraction reflects the frequency with which the corresponding codeword is used *in vivo*, and many questions related to the function of the UpUpG codeword remain unanswered. However, the data suggest that additional leucine codeword(s) will be found which may contain three different bases.[25]

Summary.—The minimum proportions of degenerate species of sRNA corresponding to one amino acid have been estimated by directing sRNA binding to ribosomes with polynucleotides. Poly UG and poly UC stimulated the stable binding of approximately 2 and 6 per cent of unfractionated C^{14}-leucine-sRNA, respectively. The binding of purified C^{14}-leucine-sRNA fraction II to ribosomes was stimulated by the trinucleotide UpUpG and by poly UG, but not by UpGpU, GpUpU, or dinucleotides. UpUpG had no effect upon the binding of two other C^{14}-leucine-sRNA fractions. Binding of S^{35}-cysteine-sRNA to ribosomes was directed by UpGpU, but not other tri- or dinucleotide sequence isomers. The nucleotide sequence UpGpU was suggested for a cysteine RNA codeword, and the sequence UpUpG for a leucine RNA codeword corresponding to leucine-sRNA fraction II.

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The following abbreviations are used: Leu-, leucine-, Phe-, phenylalanine-, Val-, valine-, and Cys-, cysteine-sRNA; poly U, polyuridylic acid; poly A, polyadenylic acid; poly UC, copolymer of uridylic and cytidylic acids; poly UG, copolymer of uridylic and guanylic acids; TCA, trichloroacetic acid; sRNA, transfer RNA. For mono- and oligonucleotides of specific structure, the "p" to the left of a terminal nucleoside initial indicates a 5'-terminal phosphate; the "p" to right, a 3'-(2')-terminal phosphate. Internal phosphates of oligonucleotides are (3',5')-linkages. Oligonucleotides whose nucleoside initials are enclosed within parentheses are of unspecified sequence.

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ON THE MOLECULAR MECHANISM OF BIOLUMINESCENCE, I. THE ROLE OF LONG-CHAIN ALDEHYDE

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It is known that the bioluminescent reaction with enzyme prepared from the bacterium *Photobacterium fischeri* proceeds via several intermediates, some of which, at least, are relatively long-lived.[1] The turnover number of 4 per min at 10°C is, indeed, apparently the lowest which has been recorded for any enzyme.

The experiments presented in this and in the paper to appear in the January issue of the PROCEEDINGS provide evidence for a new theory of the molecular mechanism of bioluminescence. It is proposed that electronic energy derived from the chemical reaction is stored in a long-lived high-energy enzyme species (designated as $\pm$Enz) and that an excited state Enz* is generated by an appropriate release of this stored energy.