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The RNA Code and Protein Synthesis

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Many properties of the RNA code which were discussed at the 1963 Cold Spring Harbor meeting were based on information obtained with randomly ordered synthetic polynucleotides. Most questions concerning the code which were raised at that time related to its fine structure, that is, the order of the bases within RNA codons. After the 1963 meetings a relatively simple means of determining nucleotide sequences of RNA codons was devised which depends upon the ability of trinucleotides of known sequence to stimulate AA-sRNA binding to ribosomes (Nirenberg and Leder, 1964). In this paper, information obtained since 1963 relating to the following topics will be discussed:

(1) The fine structure of the RNA code

(2) Factors affecting the formation of codon-ribosome-AA-sRNA complexes

(3) Patterns of synonym codons for amino acids and purified sRNA fractions

(4) Mechanism of codon recognition

(5) Universality

(6) Unusual aspects of codon recognition as potential indicators of special codon functions

(7) Modification of codon recognition due to phage infection.

FINE STRUCTURE OF THE RNA CODE

Formation of Codon-Ribosome-AA-sRNA Complexes

The assay for base sequences of RNA codons depends, first upon the ability of trinucleotides to serve as templates for AA-sRNA binding to ribosomes prior to peptide bond formation, and second, upon the observation that codon-ribosome-AA-sRNA complexes are retained by cellulose nitrate filters (Nirenberg and Leder, 1964). Results shown in Table 1 illustrate characteristics of codon-ribosome-sRNA complex formation. Ribosomes, Mg^{++}, and poly U are required for the binding of C^{14}-Phe-sRNA to ribosomes. The addition of deacylated sRNA to reactions at zero time greatly reduces the binding of C^{14}-Phe-sRNA (Table 1), since poly U specifically stimulates the binding of both deacylated $sRNA^{Phe}$ and C^{14}-Phe-sRNA to ribosomes. Ribosomal bound

Table 1. Characteristics of AA-sRNA Binding to Ribosomes

Modifications	C^{14}-Phe-sRNA bound to ribosomes ($\mu\mu$mole)
Complete	5.99
− Poly U	0.12
− Ribosomes	0.00
− Mg^{++}	0.09
+ deacylated sRNA at 50 min	
0.50 A^{260} units	5.69
2.50 A^{260} units	5.39
+ deacylated sRNA at zero time	
0.50 A^{260} units	4.49
2.50 A^{260} units	2.08

Complete reactions in a volume of 0.05 ml contained the following: 0.1 M Tris acetate (pH 7.2) (in other experiments described in this paper 0.05 M Tris acetate, pH 7.2 was used), 0.02 M magnesium acetate, 0.05 M potassium chloride (standard buffer); 2.0 A^{260} units of *E. coli* W3100 70 S ribosomes (washed by centrifugation 3 times); 15 mμmoles of uridylic acid residues of poly U; and 20.6 $\mu\mu$moles C^{14}-Phe-sRNA (0.71 A^{260} units). All components were added to tubes at 0°C. C^{14}-Phe-sRNA was added last to initiate binding reactions.

Incubation was at 0°C for 60 min (in all other experiments described in this paper, reactions were incubated at 24° for 15 min). Deacylated sRNA was added either at zero time or after 50 min of incubation, as indicated. After incubation, tubes were placed in ice and each reaction was immediately diluted with 3 ml of standard buffer at 0° to 3°C. A cellulose nitrate filter (HA type, Millipore Filter Corp., 25 mm diameter, 0.45 μ pore size) in a stainless steel holder was washed with gentle suction with 5 ml of the cold standard buffer. The diluted reaction mixture was immediately poured on the filter under suction and washed to remove unbound C^{14}-Phe-sRNA with three 3-ml and one 15-ml portions of standard buffer at 3°. Ribosomes and bound sRNA remained on the filter (Nirenberg and Leder, 1964). The filters were then dried, placed in vials containing 10 ml of a scintillation fluid (containing 4 gm 2,5-diphenyloxazole and 0.05 gm 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene) and counted in a scintillation spectrometer.

ABBREVIATIONS

The following abbreviations are used: Ala-, alanine-; Arg-, arginine-; Asn-, asparagine-; Asp-, aspartic acid-; Cys-, cysteine-; Glu-, glutamic acid-, Gln-, glutamine-, Gly-, glycine, His-, histidine-, Ile-, isoleucine-, Leu-, leucine-, Lys-, lysine-, Met-, methionine-, Phe-, phenylalanine-, Pro-, proline-, Ser-, serine-, Thr-, threonine-, Trp-, tryptophan-, Tyr-, tyrosine-, and Val-, valine-sRNA; sRNA, transfer RNA; AA-sRNA, aminoacyl-sRNA; $sRNA^{Phe}$, deacylated phenylalanine-acceptor sRNA; $Ala\text{-}sRNA^{Yeast}$, acylated alanine- acceptor sRNA from yeast. U, uridine; C, cytidine; A, adenosine; G, guanosine; I, inosine; rT, ribothymidine; ψ, pseudouridine; DiHU, dihydro-uridine; MAK, methylated albumin kieselguhr; F-Met, N-formyl-methionine. For brevity, trinucleoside diphosphates are referred to as trinucleotides. Internal phosphates of trinucleotides are (3′,5′)-phosphodiester linkages.

C^{14}-Phe-sRNA is not readily exchangeable with unbound Phe-sRNA or deacylated $sRNA^{Phe}$ except at low Mg^{++} concentrations (Levin and Nirenberg, in prep.). Later in this volume Dr. Dolph Hatfield discusses the characteristics of exchange of ribosomal bound with unbound AA-sRNA when trinucleotides are present.

Two enzymatic methods were devised for oligonucleotide synthesis, since most trinucleotide sequences had not been isolated or synthesized earlier. One procedure employed polynucleotide phosphorylase to catalyze the synthesis of oligonucleotides from dinucleoside monophosphate primers and nucleoside diphosphates (Leder, Singer, and Brimacombe, 1965; Thach and Doty, 1965); the other approach (Bernfield, 1966) was based upon the demonstration (Heppel, Whitfeld, and Markham, 1955) that pancreatic RNase catalyzes the synthesis of oligonucleotides from uridine- or cytidine-2′,3′-cyclic phosphate and acceptor moieties. Elegant chemical procedures for oligonucleotide synthesis devised by Khorana and his associates (see Khorana et al., this volume) also are available.

Template Activity of Oligonucleotides with Terminal and Internal Substitutions

The trinucleotides, UpUpU and ApApA, but not the corresponding dinucleotides, stimulate markedly the binding of C^{14}-Phe- and C^{14}-Lys-sRNA, respectively. Such data directly demonstrate a triplet code and also show that codons contain three *sequential* bases. The template activity of triplets with 5′-terminal phosphate, pUpUpU, equals that of the corresponding tetra- and pentanucleotides; whereas, oligo U preparations with 2′,3′-terminal phosphate are much less active. Hexa-A preparations, with and without 3′-terminal phosphate, are considerably more active as templates than the corresponding pentamers; thus, one molecule of hexa-A may be recognized by two Lys-sRNA molecules bound to adjacent ribosomal sites (Rottman and Nirenberg, 1966).

An extensively purified doublet with 5′-terminal phosphate, pUpC, serves as a template for Ser-sRNA (but not for Leu- or Ile-sRNA), whereas a doublet without terminal phosphate, UpC, is inactive (see Figs. 1a and b). However, the template activity of pUpC is considerably lower than that of the triplet, UpCpU. The relation between Mg^{++} concentration and template activity is shown in Fig. 1b. pUpC and UpCpU stimulate Ser-sRNA binding in reactions containing 0.02–0.08 M Mg^{++}. These results demonstrate that a doublet with 5′-terminal phosphate can serve as a specific, although relatively weak, template for AA-sRNA. It is particularly intriguing to relate recognition of a doublet to the

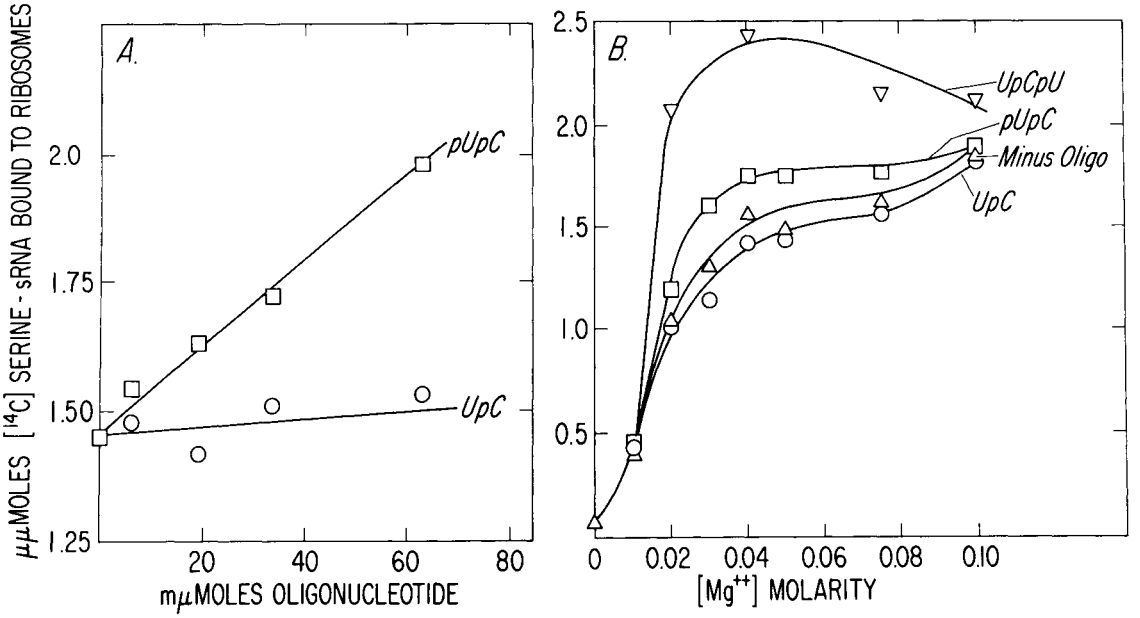
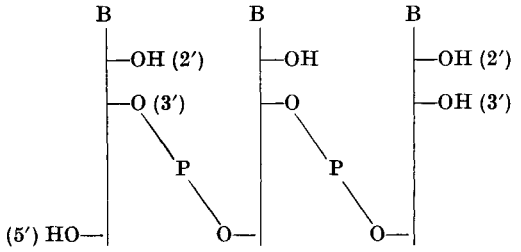


Figure 1a, b. The effects of UpC and pUpC on the binding of C^{14}-Ser-sRNA to ribosomes. The relation between oligonucleotide concentration and C^{14}-Ser-sRNA binding to ribosomes at 0.03 M Mg^{++} is shown in Fig. 1a. It should be noted that the ordinate begins at 1.25 $\mu\mu$moles of C^{14}-Ser-sRNA. The relation between Mg^{++} concentration and C^{14}-Ser-sRNA binding to ribosomes is shown in Fig. 1b. As indicated, 50 mμmoles of UpC or pUpC, or 15 mμmoles of UpCpU, were added to each reaction. Each point in parts a and b represents a 50 μl reaction containing the components described in the legend to Table 1 except for the following: 14.3 $\mu\mu$moles C^{14}-Ser-sRNA (0.42 A^{260} units); 1.1 A^{260} units of ribosomes. Incubations were for 15 min at 24°C. (Data from Rottman and Nirenberg, 1966.)

TABLE 2. RELATIVE TEMPLATE ACTIVITY OF SUBSTITUTED OLIGONUCLEOTIDES



Oligonucleotide	Relative template activity
p-5′-UpUpU	510
UpUpU	100
CH_3O-pUpUpU	74
UpUpU-3′3-p	48
UpUpUp-OCH_3	18
UpUpU-2′,3′-cyclic p	17
(2′-5′)-UpUpU	0
Oligodeoxy T	0
p-5′-ApApA	181
ApApA	100
ApApA-3′-p	57
ApApA-2′-p	15
(2′-5′)-ApApA	0
Oligodeoxy A	0

Relative template activities are approximations obtained by comparing the amount of AA-sRNA bound to ribosomes in the presence of limiting concentrations of oligonucleotides (0.50 or 0.12 mμmoles of oligonucleotides containing U or A, respectively) compared to either UpUpU, for C^{14}-Phe-sRNA; or ApApA, for C^{14}-Lys-sRNA (each assumed to be 100%). Data are from Rottman and Nirenberg (1966) except results with oligodeoxynucleotides which are from Nirenberg and Leder (1964).

possibility that only two out of three bases in a triplet may be recognized occasionally during protein synthesis, and also to the possibility that a triplet code evolved from a more primitive doublet code.

Further studies on template activities of oligonucleotides with terminal and internal modifications are summarized in Table 2. At limiting oligonucleotide concentrations, the relative template activities of oligo U preparations are as follows: p-5′-UpUpU > UpUpU > CH_3O-p-5′-UpUpU > UpUpU-3′-p > UpUpU-3′-p-OCH_3 > UpUpU-2′,3′-cyclic phosphate. Trimers with (2′-5′) phosphodiester linkages, (2′-5′)-UpUpU and (2′-5′)-ApApA, do not serve as templates for Phe- or Lys-, sRNA respectively. The relative template efficiencies of oligo A preparations are as follows: p-5′-ApApA > ApApA > ApApA-3′-p > ApApA-2′-p.

These studies led to the proposal that RNA and DNA contain three classes of codons, differing in structure; 5′-terminal, 3′-terminal, and internal codons (Nirenberg and Leder, 1964). Certainly the first base of a 5′-terminal codon and the third base of a 3′-terminal codon may be recognized with less fidelity than an internal codon, for in the absence of a nucleotide neighbor a terminal base may have a greater freedom of movement on the ribosome. Substitution of 5′- or 3′-terminal hydroxyl groups may impose restrictions upon the orientation of terminal bases during codon recognition. 5′-Terminal and perhaps also 3′-terminal codons possibly serve, together with neighboring codons, as operator regions.

Since many enzymes have been described which catalyze the transfer of nucleotides, amino acids, phosphate, and other molecules to or from terminal ribose or deoxyribose of nucleic acids, modification of sugar hydroxyl groups was proposed as a possible mechanism for regulating the reading of RNA or DNA (Nirenberg and Leder, 1964).

NUCLEOTIDE SEQUENCES OF RNA CODONS

A summary of nucleotide sequences of RNA codons by *E. coli* AA-sRNA is shown in Table 3

TABLE 3. NUCLEOTIDE SEQUENCES OF RNA CODONS

1st Base	2nd Base U	2nd Base C	2nd Base A	2nd Base G	3rd Base
U	PHE*	SER*	TYR*	CYS*	U
	PHE*	SER*	TYR*	CYS	C
	leu*?	SER	TERM?	cys?	A
	leu*, f-met	SER*	TERM?	TRP*	G
C	leu*	pro*	HIS*	ARG*	U
	leu*	pro*	HIS*	ARG*	C
	leu	PRO*	GLN*	ARG*	A
	LEU	PRO	gln*	arg	G
A	ILE*	THR*	ASN*	SER	U
	ILE*	THR*	ASN*	SER*	C
	ile*	THR*	LYS*	arg*	A
	MET*, F-MET	THR	lys	arg	G
G	VAL*	ALA*	ASP*	GLY*	U
	VAL	ALA*	ASP*	GLY*	C
	VAL*	ALA*	GLU*	GLY*	A
	VAL	ALA	glu	GLY	G

Nucleotide sequences of RNA codons were determined by stimulating binding of *E. coli* AA-sRNA to *E. coli* ribosomes with trinucleotide templates. Amino acids shown in capitals represent trinucleotides with relatively high template activities compared to other trinucleotide codons corresponding to the same amino acid. Asterisks (*) represent base compositions of codons which were determined previously by directing protein synthesis in *E. coli* extracts with synthetic randomly-ordered polynucleotides (Speyer et al., 1963; Nirenberg et al., 1963). F-Met, represents N-formyl-Met-sRNA which may recognize initiator codons. TERM represents possible terminator codons. Question marks (?) indicate uncertain codon function. Data are from Nirenberg et al., 1965; Brimacombe et al., 1965; also see articles by Khorana et al., Söll et al., and Matthaei et al., in this volume.

TABLE 4. PATTERNS OF DEGENERATE CODONS FOR AMINO ACIDS

●●U ●●C	●●A ●●G	●●U ●●C ●●A ●●G	●●U ●●C ●●(A)	●●U ●●C	●●A ●●G	●●G	●●U ●●C ●●A ●●(G)
SER	ARG LEU	GLY ALA VAL THR PRO	CYS ILE	ASP ASN HIS TYR PHE	GLU GLN LYS TERM?	MET TRP	F-MET

Solid circles represent the first and second bases of trinucleotides; U, C, A, and G indicate bases which may occupy the remaining position of degenerate codons. In the case of F-Met (N-formylmethionine), circles represent the second and third bases. Parentheses indicate codons with relatively low template activities.

and patterns of degeneracy in Table 4. Almost every trinucleotide was assayed for template specificity with 20 AA-sRNA preparations (unfractionated sRNA acylated with one labeled and 19 unlabeled amino acids). It is important to test trinucleotide template specificity with 20 AA-sRNA preparations, since relative responses of AA-sRNA are then quite apparent. In surveying trinucleotide specificity, unfractionated AA-sRNA should be used initially because altering ratios of sRNA species often influences the fidelity of codon recognition.

Almost all triplets correspond to amino acids; furthermore, patterns of codon degeneracy are logical. Six degenerate codons correspond to serine, five or six to arginine and also to leucine, and from one to four to each of the remaining amino acids. Alternate bases often occupy the third positions of triplets comprising degenerate codon sets. In all cases triplet pairs with 3′-terminal pyrimidines (XYU and XYC, where X and Y represent the first and second bases, respectively, in the triplet) correspond to the same amino acid; often XYA and XYG correspond to the same amino acid; sometimes XYG alone corresponds to an amino acid. For eight amino acids, U, C, A, or G may occupy the third position of synonym codons. Alternate bases also may occupy the first position of synonyms, as for N-formyl-methionine.

One consequence of logical degeneracy is that many single base replacements in DNA may be silent and thus not result in amino acid replacement in protein (cf. Sonneborn, 1965). Also, the code is arranged so that the effects of some errors may be minimized, since amino acids which are structurally or metabolically related often correspond to similiar RNA codons (for example, Asp-codons, GAU, and GAC, are similar to Glu-codons, GAA, and GAG). When various amino acids are grouped according to common biosynthetic precursors, close relationships among their synonym codons sometimes are observed. For example, codons for amino acids derived from aspartic acid begin with A: Asp, GAU, GAC; Asn, AAU, AAC; Lys, AAA, AAG; Thr, ACU, ACC, ACA, ACG; Ile, AUU, AUC, AUA; Met, AUG. Likewise, aromatic amino acids have codons beginning with U; Phe, UUU, UUC; Tyr, UAU, UAC; Trp, UGG. Such relationships may reflect either the evolution of the code or direct interactions between amino acids and bases in codons (see Woese et al., this volume).

At the time of the 1963 meeting at Cold Spring Harbor, 53 base compositions of RNA codons had been estimated (14 tentatively) in studies with randomly-ordered synthetic polynucleotides and a cell-free protein synthesizing system derived from *E. coli* (Speyer et al., 1963; Nirenberg et al., 1963). Forty-six base composition assignments now are confirmed by base sequence studies with trinucleotides (shown in Table 3). Thus, codon base compositions and base sequence assignments, obtained by assaying protein synthesis and AA-sRNA binding, respectively, agree well with one another. In addition, codon base sequences are confirmed by most amino acid replacement data obtained in vivo (see Yanofsky et al.; Wittman et al., this volume).

PATTERNS OF SYNONYM CODONS RECOGNIZED BY PURIFIED sRNA FRACTIONS

Table 5 contains a summary of synonym codons recognized by purified sRNA fractions obtained either by countercurrent distribution or by MAK column chromatography. The following patterns of codon recognition involving alternate bases in the third positions of synonym codons were found; C = U; A = G; G; U = C = A; A = G = (U). For example, Val-sRNA$_3$ recognizes GUU and GUC, whereas the major peak of Val-sRNA (fractions 1 and 2) recognizes GUA, GUG and, to a lesser extent, GUU. The possibility that the latter Val-sRNA fraction contains two or more Val-sRNA components has not been excluded. Met-sRNA$_1$

TABLE 5. CODON PATTERNS RECOGNIZED BY PURIFIED sRNA FRACTIONS

Alternate acceptable bases in 3rd or 1st positions of triplet					
C U	A G	G	U C A	A G (U)	Possibly only 2 bases recognized
$TYR_{1,2}$ UA(C,U)	LYS AA(A,G)	LEU_2 CUG	ALA^{yeast} GCC U,A	ALA_1 GCG A,(U)	LEU_3 CU(U,C)
VAL_3 GU(C,U)		LEU_5 UUG	$SER^{yeast}_{2,3}$ UCC U,A	$VAL_{1,2}$ GUG A,(U)	$LEU_{4a,b}$ UU(U,C)
		MET_2 AUG	F-MET_1 UG U,C,A		LEU_1 (U)UG
			TRP_2 CGG U,(A)		

Patterns of degenerate codons recognized by purified AA-sRNA fractions. sRNA fractions are from *E. coli* B, unless otherwise specified. At the top of the table are shown the alternate bases which may occupy the third or first positions of degenerate codon sets. Purified sRNA fractions and corresponding codons are shown below. Parentheses indicate codons with relatively low template activity. sRNA fractions were obtained by counter-current distribution (Kellogg et al., 1966), unless otherwise specified. Yeast Ser-sRNA fractions 2 and 3 (Connelly and Doctor, 1966) are thought to be equivalent to yeast Ser-sRNA fractions 1 and 2, respectively, discussed by Zachau et al. in this volume. Yeast Ala-sRNA was the gift of R. W. Holley; results are from Leder and Nirenberg (unpubl.). Results obtained with Val-, Met-, and Ala-$sRNA^{E.\ coli}$ fractions are from Kellogg et al. (1966). For additional results with Tyr-sRNA fractions, see Doctor, Loebel and Kellogg, this volume. Leu-sRNA fractions (see Fig. 6 and Sueoka et al., this volume) and Lys-sRNA (Kellogg, Doctor, and Nirenberg, unpubl.) were obtained by MAK column chromatography. Three Leu-sRNA fractions also were obtained by counter-current distribution (Nirenberg and Leder, 1964). Reactions contained the usual components (see legend to Table 1) and 0.01 or 0.02 M Mg^{++}. Incubation was at 24° for 15 min.

responds to UUG, CUG, AUG and, to a lesser extent, GUG, and can be converted enzymatically to N-formyl-Met-sRNA, whereas, Met-$sRNA_2$ responds primarily to AUG and does not accept formyl moieties (see later discussion). Unfractionated Trp-sRNA responds only to UGG; however one fraction of Trp-sRNA, after extensive purification, responds to UGG, CGG and AGG. Possibly the latter responses depend upon the removal of sRNA for other amino acids (e.g., Arg-sRNA) which also may recognize CGG or AGG. Yeast Ala- and Ser-$sRNA_{2,3}$ fractions recognize synonyms containing U, C, or A in the third position. Leu-$sRNA_{1,3,4}$ bind to ribosomes in response to polynucleotide templates but not to trinucleotides. Possibly, only two of the three bases are recognized by these Leu-sRNA fractions.

MECHANISM OF CODON RECOGNITION

Crick (1966; also this volume) has suggested that certain bases in anticodons may form alternate hydrogen bonds, via a wobble mechanism, with corresponding bases in mRNA codons. This hypothesis and further experimental findings are discussed below.

Yeast Ala-sRNA of known base sequence and of high purity (>95%) was the generous gift of Dr. Robert Holley. In Figs. 2 and 3 are shown the responses of purified yeast and unfractionated *E. coli* C^{14}-Ala-sRNA, respectively, to synonym Ala-codons as a function of Mg^{++} concentration. Purified yeast C^{14}-Ala-sRNA responds well to GCU, GCC, and GCA, but only slightly to GCG. Similar results were obtained with unfractionated Ala-$sRNA^{Yeast}$. In contrast, unfractionated *E. coli* C^{14}-Ala-sRNA responds best to GCG and GCA, less well to GCU, and only slightly to GCC.

In Fig. 4a and b, the relation between concentration of yeast or *E. coli* C^{14}-Ala-sRNA and response to synonym Ala-codons is shown. At *limiting* concentrations of purified yeast C^{14}-Ala-sRNA, at least 59, 45, 45, and 3% of the available C^{14}-Ala-sRNA molecules bind to ribosomes in response to GCU, GCC, GCA, and GCG, respectively. The response of unfractionated *E. coli* C^{14}-Ala-sRNA to each codon was 18, 2, 38, and 64%, respectively. Similar results have been obtained by Keller and Ferger (1966) and Söll et al. (this volume). Since the purity of the yeast Ala-sRNA was greater than 95%, the extent of binding at limiting Ala-sRNA concentrations indicates that one molecule of Ala-sRNA recognizes 3, possibly 4, synonym codons. In addition, the data demonstrate marked differences between the relative responses of yeast and *E. coli* Ala-sRNA to synonym codons.

Correlating the base sequences of yeast Ala-sRNA with corresponding mRNA codons also provides insight into the structure of the Ala-sRNA

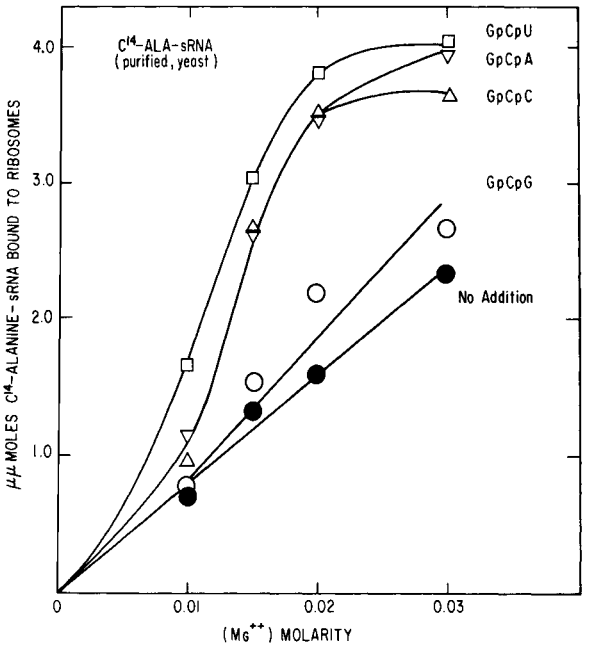


FIGURE 2. The relation between Mg^{++} concentration and binding to ribosomes of purified yeast C^{14}-Ala-sRNA of known base sequence (Holley et al., 1965) in response to trinucleotides. Each point represents a 50 μl reaction containing the components described in the legend to Table 1 except for the following: 1.5 A^{260} units of *E. coli* ribosomes, 11.2 $\mu\mu$moles of purified yeast C^{14}-Ala-sRNA (0.038 A^{260} units); and 0.1 A^{260} units of trinucleotide as specified. Reactions were incubated at 24° for 15 min (Leder and Nirenberg, unpubl.).

anti-codon and the mechanism of codon recognition. Possible anticodon or enzyme recognition sequences in Ala-$sRNA^{Yeast}$ are –IGC MeI– and DiHU–CGG–DiHU (Fig. 5; Holley et al., 1965). Each site potentially comprises a single-stranded loop region at the end of a hairpin-like double-stranded segment. If CGG were the anticodon, *parallel* hydrogen bonding with GCU, GCC, GCA codons would be expected. If IGC were the anticodon, *antiparallel* Watson-Crick hydrogen bonding between GC in the anticodon and GC in the first and second positions of codons, and alternate pairing of inosine in the anticodon with U, C, or A, but not G, in the third position of Ala-codons, would be expected. All of the available evidence is consistent with an IGC Ala-anticodon. Zachau has shown that Ser-$sRNA^{Yeast}_{1 \text{ and } 2}$ contain, in appropriate positions, IGA sequences (Zachau, Dütting, and Feldmann, 1966), and we find that Ser-$sRNA^{Yeast}$ fractions 2 and 3 (believed to correspond to fractions 1 and 2 of Zachau) recognize UCU, UCC, and UCA, but not UCG (see Table 5). A purified Val-$sRNA^{Yeast}$ fraction contains the sequence IAC which corresponds to three Val-codons, GUU, GUC, and GUA (Ingram and Sjöqvist, 1963). In addition, the sequence, GψA, is found at the postulated anticodon site of Tyr-$sRNA^{Yeast}$ which corresponds to the Tyr-codons, UAU and UAC (Madison, Everett, and Kung, 1966).

Crick's wobble hypothesis and patterns of synonym codons found experimentally are in full agreement. In Table 6 are shown bases in anticodons which form alternate hydrogen bonds, via the wobble mechanism, with bases usually occupying the third positions of mRNA codons. U in the sRNA anticodon may pair alternately with A or G in mRNA codons; C may pair with G; A with U; G with C or U; and I with U, C, or A. In addition, we suggest that ribo T in the anticodon may hydrogen bond more strongly with A, and perhaps with G also, than U; and ψ in the anticodon may hydrogen bond alternately with A, G or, less well, U.

Dihydro U in an anticodon may be unable to hydrogen bond with a base in mRNA but may be repelled less by pyrimidines than by purines.

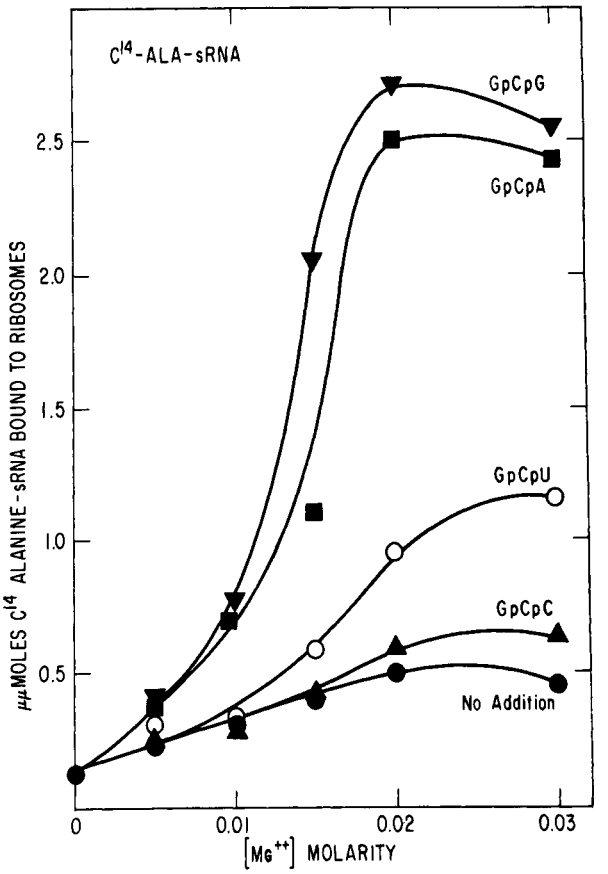


FIGURE 3. Relation between Mg^{++} concentration and binding of unfractionated *E. coli* C^{14}-Ala-sRNA to ribosomes in response to trinucleotides. Each point represents a 50 μl reaction containing the components described in the legend to Table 1, 2.0 A^{260} units of ribosomes; 18.8 $\mu\mu$moles of unfractionated *E. coli* C^{14}-Ala-sRNA (0.54 A^{260} units); and 0.1 A^{260} unit of trinucleotide, as specified (Leder and Nirenberg, unpubl.).

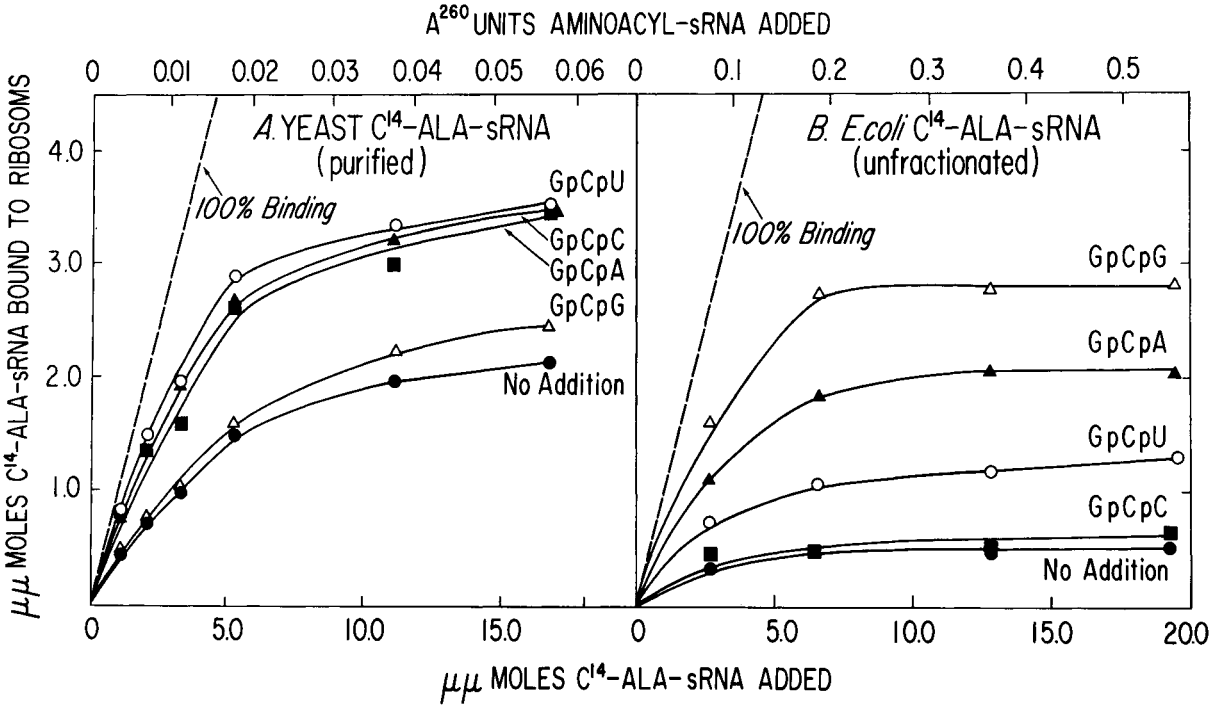


FIGURE 4a, b. Relation between the template activities of trinucleotides and the concentrations of purified yeast C^{14}-Ala-sRNA (part a) and unfractionated *E. coli* C^{14}-Ala-sRNA (part b). Each point represents a 50 μl reaction containing the components described in the legend of Table 1, and the following components: 0.02 M magnesium acetate; 0.1 A^{260} unit of trinucleotide as specified; 1.1 A^{260} units of *E. coli* ribosomes (part a) and 2.0 A^{260} units of *E. coli* ribosomes (part b); and C^{14}-Ala-sRNA as indicated on the abscissa (Leder and Nirenberg, unpubl.).

Possibly, hydrogen bonds then form between the two remaining bases of the codon (bases 1 and 2, or 2 and 3) and the corresponding bases in the anticodon. Only two out of three bases in a codon would then be recognized. This possibility is supported by the studies of Rottman and Cerutti (1966) and Cerutti, Miles, and Frazier, (1966). Possibly, some synonym codon patterns may be due to the formation of two rather than three base pairs per triplet, particularly if both are

RECOGNITION OF ALA-CODONS BY YEAST ALA-sRNA

	ME	Di H Di H
sRNA	CUUIGCIψGG	UAGUCGGUAGC
mRNA	GCU	GCU
	GCC	GCC
	GCA	GCA
	(GCG)	(GCG)

FIGURE 5. Base sequences from yeast Ala-sRNA shown in the upper portion of the figure represent possible anticodons. Base sequences of synonym RNA Ala-codons are shown in the lower portion of the figure. The first and second bases of Ala-codons on the left would form antiparallel Watson-Crick hydrogen bonds with the anticodon, while those on the right would form parallel hydrogen bonds. See text for further details.

TABLE 6. ALTERNATE BASE PAIRING

sRNA Anticodon	mRNA Codon
U	A G
C	G
A	U
G	C U
I	U C A
rT	A G
ψ	A G (U)
DiHU	No base pairing

The base in an sRNA anticodon shown in the left-hand column forms antiparallel hydrogen bonds with the base(s) shown in the right-hand column, which usually occupy the third position of degenerate mRNA codons. Relationships for U, C, A, G, and I of anticodons are "wobble" hydrogen bonds suggested by Crick (1966; also this volume). See text for further details.

TABLE 7. NUCLEOTIDE SEQUENCES OF RNA CODONS RECOGNIZED BY AA-sRNA FROM BACTERIA AND AMPHIBIAN AND MAMMALIAN LIVER

	U	C	A	G	
U	PHE	SER	TYR	cys	U
	PHE	SER	TYR	cys	C
	leu?	SER	TERM?	[cys]	A
	leu, F-MET	[SER]	TERM?	trp	G
C	leu	PRO	HIS	ARG	U
	leu	PRO	HIS	ARG	C
	leu	PRO	gln	ARG	A
	leu	PRO	gln	[ARG]	G
A	ILE	THR	asn	[SER]	U
	ILE	THR	asn	[SER]	C
	[ILE]	THR	LYS	[ARG†]	A
	MET, F-MET?	THR	[LYS]	[ARG]	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	gly	A
	VAL	[ALA]	GLU	gly	G

Universality of the RNA code. Nucleotide sequences and relative template activities of RNA codons determined with trinucleotides and AA-sRNA from *E. coli*, *Xenopus laevis* and guinea pig liver. Rectangles represent trinucleotides which are active templates for AA-sRNA from one organism, but not from another. Assignments in capitals indicate that the trinucleotide was assayed with AA-sRNAs from *E. coli*, *Xenopus laevis* liver, and guinea pig liver. Assignments in lower case indicate that the trinucleotide was assayed only with *E. coli* AA-sRNA (with the exception of cys-codons which were assayed with both *E. coli* and guinea pig liver Cys-sRNA).

†Söll et al. (1965) reported that both AGA and AGG stimulate yeast Arg-sRNA binding to ribosomes. The trinucleotide, AGA, however, has little or no effect upon the binding of *E. coli*, *Xenopus laevis* or guinea pig Arg-sRNA to ribosomes.

Reactions contained components described in the legend to Table 1, 0.01 or 0.02 M Mg^{++}, *E. coli* ribosomes, and 0.150 A^{260} units of trinucleotides (data from Marshall, Caskey, and Nirenberg, in prep.).

(C) · (G) pairs (also see earlier discussion concerning template activity of pUpC).

In summary, patterns for amino acids often represent the sum of two or more codon patterns recognized by different sRNA species. Specific sRNA patterns, in turn, often result from alternate pairing between bases in the codon and anticodon or, possibly, from the formation of only two base pairs if the remaining bases do not greatly repel one another.

UNIVERSALITY

The results of many studies indicate that the RNA code is largely universal. However, translation of the RNA code can be altered in vivo by extragenic suppressors and in vitro by altering components of reactions or conditions of incubation. Thus, cells sometimes differ in specificity of codon translation.

To investigate the fine structure of the code recognized by AA-sRNA from different organisms, nucleotide sequences and relative template activities of RNA codons recognized by bacterial, amphibian, and mammalian AA-sRNA (*E. coli*, *Xenopus laevis* and guinea pig liver, respectively) were determined (Marshall, Caskey, and Nirenberg, submitted for publication). Acylation of sRNA was catalyzed in all cases by aminoacyl-sRNA synthetases from corresponding organisms and tissues. *E. coli* ribosomes were used for binding studies. Therefore, the specificities of sRNA and AA-sRNA synthetases were investigated.

The results are shown in Table 7. Almost identical translations of nucleotide sequences to amino acids were found with bacterial, amphibian, and mammalian AA-sRNA. In addition, similar sets of synonym codons usually were recognized by AA-sRNA from each organism. However, *E. coli* AA-sRNA sometimes differed strikingly from *Xenopus* and guinea pig liver AA-sRNA in relative response to synonym codons. Differences in codon recognition are shown in Table 8. The following

TABLE 8. SPECIES DEPENDENT DIFFERENCES IN RESPONSE OF AA-sRNA TO TRINUCLEOTIDE CODONS

		sRNA		
Codon		Bacterial (*E. coli*)	Amphibian (*Xenopus laevis*)	Mammalian (Guinea pig liver)
ARG	AGG	±	++++	+++
	CGG	±	++++	++++
MET	UUG	++	±	±
ALA	GCG	++++	±	++
ILE	AUA	±	++	++
LYS	AAG	±	++++	++++
SER	UCG	++++	±	++
	AGU	±	+++	+++
	AGC	±	+++	+++
CYS	UGA	±		+++

Possible differences: ACG, THR; AUC, ILE; CAC, HIS; GUC, VAL; and GCC, ALA

No differences found: ASP, GLY, GLU, PHE, PRO, and TYR.

The following scale indicates the approximate response of AA-sRNA to a trinucleotide relative to the responses of the same AA-sRNA preparation to all other trinucleotides for that amino acid (except Gly-sRNA which was assayed only with GGU and GGC).

++++	70–100%
+++	50–70%
++	20–50%
±	0–20%

trinucleotides had little or no detectable template activity for unfractionated *E. coli* AA-sRNA but served as active templates with *Xenopus* and guinea pig AA-sRNA: AGG, CGG, arginine; AUA, isoleucine; AAG, lysine; AGU, AGC, serine; and UGA, cysteine. Those trinucleotides with high template activity for *E. coli* AA-sRNA but low activity for *Xenopus* or guinea pig liver AA-sRNA were: UUG, N-formyl-methionine; GCG, alanine; and UCG, serine. Possible differences also were observed with ACG, threonine; AUC, isoleucine; CAC, histidine; GCC, alanine; and GUC, valine. No species dependent differences were found with Asp-, Gly-, Glu-, Phe-, Pro-, and Tyr-codons.

Thus, some degenerate trinucleotides were active templates with sRNA from each species studied, whereas others were active with sRNA from one species but not from another.

UAA and UAG do not appreciably stimulate binding of unfractionated *E. coli* AA-sRNA (AA-sRNA for each amino acid tested); *Xenopus* Arg-, Phe-, Ser-, or Tyr-sRNA; or guinea pig Ala-, Arg-, Asp-, His-, Ile-, Met-, Pro-, Ser-, or Thr-sRNA.

Nucleotide sequences recognized by *Xenopus* skeletal muscle Arg-, Lys-, Met-, and Ser-sRNA were determined and compared with sequences recognized by corresponding *Xenopus* liver AA-sRNA preparations. No differences between liver and muscle AA-sRNA were detected, either in nucleotide sequences recognized or in relative responses to synonym codons.

Fossil records of bacteria 3.1 billion years old have been reported (Barghoorn and Schopf, 1966). The first vertebrates appeared approximately 510 million years ago, and amphibians and mammals, 355 and 181 million years ago, respectively. The presence of bacteria 3 billion years ago may indicate the presence of a functional genetic code at that time. Almost surely the code has functioned for more than 500 million years. The remarkable similarity in codon base sequences recognized by bacterial, amphibian, and mammalian AA-sRNA suggest that most, if not all, forms of life on this planet use almost the same genetic language, and that the language has been used, possibly with few major changes, for at least 500 million years.

UNUSUAL ASPECTS OF CODON RECOGNITION AS POTENTIAL INDICATORS OF SPECIAL CODON FUNCTIONS

Most codons correspond to amino acids; however, some codons serve in other capacities, such as initiation, termination or regulation of protein synthesis. Although only a few codons have been assigned special functions thus far, we think it likely that many additional codons eventually may be found to serve special functions. Unusual properties of codon recognition sometimes may indicate special codon functions. For example, the properties of initiator and terminator codons, during codon recognition, are quite distinctive (see below). We find that approximately 20 codons have unusual properties related either to codon position, template activity, specificity, patterns of degeneracy, or stability of codon-ribosome-sRNA complexes. Until more information is available these observations will be considered as *possible* indicators of special codon functions.

Conclusions will be stated first to provide a frame of reference for discussion:

(1) A codon may have alternate meanings. (For example, UUG at or near the 5′-terminus of mRNA may correspond to N-formyl-methionine; whereas, an internal UUG codon may correspond to leucine.)

(2) A codon may serve multiple functions simultaneously. (For example, a codon may specify both initiation and an amino acid, perhaps via AA-sRNA with high affinity for peptidyl-sRNA sites on ribosomes.)

(3) Codon function sometimes is subject to modification.

(4) Degenerate codons for the same amino acid often differ markedly in template properties.

Codon Frequency and Distribution

Often, multiple species of sRNA corresponding to the same amino acid recognize different synonym codons. Degenerate codon usage in mRNA sometimes is nonrandom (Garen, pers. comm.; also von Ehrenstein; Weigert et al., this volume). The possibility that different sets of sRNA may be required for the synthesis of two proteins with the same amino acid composition suggests that protein synthesis sometimes may be regulated by codon frequency and distribution coupled with differential recognition of degenerate codons. Possibly, the rates of synthesis of certain proteins may be regulated simultaneously by alterations which affect the apparatus recognizing one degeneracy but not another (see reviews by Ames and Hartman, 1963; and Stent, 1964).

Codon Position

As discussed in an earlier section, the template properties of 5′-terminal-, 3′-terminal-, and internal- codons may differ. Regulatory mechanisms based on such differences have been suggested. Reading of mRNA probably is initiated at or near the 5′-terminal codon and then proceeds toward the 3′-terminus of the RNA chain (Salas, Smith,

Stanley, Jr., Wahba, and Ochoa, 1965). It is not known whether mechanisms of 5′-terminal and internal initiation in polycistronic messages are similar. Also, internal- and 3′-terminal mechanisms of termination remain to be defined.

N-formyl-Met-sRNA may serve as an initiator of protein synthesis in *E. coli* (Clark and Marcker, 1966; Adams and Capecchi, 1966; Webster, Englehardt, and Zinder, 1966; Thach, Dewey, Brown, and Doty, 1966). Met-sRNA$_1$ can be converted enzymatically to N-formyl-Met-sRNA$_1$ and responds to UUG, CUG, AUG and, to a lesser extent, GUG. Met-sRNA$_2$ does not accept formyl-moieties and responds primarily to AUG (Clark and Marcker, 1966; Marcker et al., this volume; also Kellogg, Doctor, Loebel, and Nirenberg, 1966). In *E. coli* extracts protein synthesis is initiated in at least two ways: by initiator codons specifying N-formyl-Met-sRNA or, at somewhat higher Mg^{++} concentrations, by another means, probably not dependent upon N-formyl-Met-sRNA since many synthetic polynucleotides without known initiator codons direct cell-free protein synthesis (Nakamoto and Kolakofsky, 1966). Poly U, for example, directs di- as well as polyphenylalanine synthesis (Arlinghaus, Schaeffer, and Schweet, 1964). Probably codons for N-formyl-Met-sRNA initiate protein synthesis with greater accuracy than codons which serve as initiators only at relatively high Mg^{++} concentrations.

UAA and UAG may function as terminator codons (Brenner, Stretton, and Kaplan, 1965; Weigert and Garen, 1965). The trinucleotides UAA and UAG do not stimulate binding appreciably of *unfractionated E. coli* AA-sRNA to ribosomes. However, sRNA fraction(s) corresponding to UAA and/or UAG are not ruled out.

Extragenic suppressors may affect the specificity of UAA and/or UAG recognition (see review by Beckwith and Gorini, 1966). The efficiencies of ochre suppressors (UAA) are relatively low compared to that of amber suppressors (UAG). Since amber suppressors do not markedly affect the rate of cell growth, and ochre suppressors with high efficiency have not been found, UAA may specify chain termination in vivo more frequently than UAG. In a study of great interest, Newton, Beckwith, Zipser and Brenner (1965) have shown that the synthesis of protein (probably mRNA also) is regulated by the relative position in the RNA message of codons sensitive to amber suppressors. Therefore, a codon may perform a regulatory function at one position but not at another.

Template Activity

Trinucleotides with little activity for AA-sRNA (in studies thus far) are: UAA, UAG, and UUA, (perhaps CUA also). In addition, the following trinucleotides are active templates with AA-sRNA from one organism, but not from another: AGG, AGA, CGG, arginine; UUG, (N-formyl-)-methionine; GCG, alanine; AUA, isoleucine; AAG, lysine, UCG, AGU, AGC, serine; and UGA, cysteine (see Universality Section and Table 9). However, some inactive trinucleotides possibly function as active codons at internal positions. For example, the following codon base compositions were estimated with synthetic polynucleotides and a cell-free protein synthesizing system from *E. coli*; AUA, isoleucine; AGA, arginine; and AGC, serine (Nirenberg et al., 1963; Speyer et al., 1963; also see Jones, Nishimura, and Khorana, 1966, for results with AGA). Among the many possible explanations for low template activities of trinucleotides in binding assays are: special codon function; codon position; appropriate species of sRNA absent or in low concentration; competition for codons or for ribosomal sites by additional species of sRNA; high ratio of deacylated to AA-sRNA; cryptic (non-acylatable) sRNA; reaction conditions, e.g., low concentration of Mg^{++} or other components, time or temperature of incubation.

Codon Specificity

Often synonym trinucleotides differ strikingly in template specificity. Such observations may indicate that template specificities of terminal- and internal-codons differ, or that special function codons or suppressors are present. At 0.010–0.015 M Mg^{++}, trinucleotide template specificity is high, in many cases higher than that of a polynucleotide; for example, poly U, but not UUU, stimulates binding of Ile-sRNA to ribosomes. However, at 0.03 M Mg^{++} ambiguous recognitions of tri- and polynucleotides are observed more frequently.

Relative template activities of synonym trinucleotides in reactions containing 0.01 or 0.03 M Mg^{++} are shown in Table 9. In some cases, only one or two trinucleotides in a synonym set are active templates at 0.01 M Mg^{++}; whereas all degeneracies are active at 0.03 M Mg^{++} (e.g., Glu, Lys, Ala, Thr). In other cases either all synonym trinucleotides are active at 0.01 M Mg^{++} as well as at 0.03 M Mg^{++} (e.g., Val), or none are active at the lower Mg^{++} concentration (e.g., Tyr, His, Asn). Such data suggest that codon-ribosome-AA-sRNA complexes formed with degenerate trinucleotides often differ in stability.

MODIFICATION OF CODON RECOGNITION DUE TO PHAGE INFECTION

N. and T. Sueoka (1964; also see Sueoka et al., this volume) have shown that infection of *E. coli*

TABLE 9. TEMPLATE ACTIVITY OF TRINUCLEOTIDES IN 0.01 OR 0.03 M Mg^{++}

	U	C	A	G	
	PHE	[SER]	TYR	[CYS]	U
	PHE	SER	TYR	[CYS]	C
U					
		(SER)			A
	[F-MET]	[SER]		(TRP)	G
		PRO	HIS	[ARG]	U
		PRO	HIS	ARG	C
C					
		(PRO)	GLN	[ARG]	A
	LEU	(PRO)	[GLN]	ARG	G
	ILE	[THR]	ASN	SER, CYS	U
	ILE	THR	ASN	SER, CYS	C
A					
		THR	[LYS]		A
	[MET]	[THR]	LYS		G
	[VAL]	ALA	[ASP]	[GLY]	U
	[VAL]	ALA	[ASP]	[GLY]	C
G					
	[VAL]	[ALA]	[GLU]	(GLY)	A
	[VAL]	[ALA]	GLU	(GLY)	G

Legend:	0.01 M Mg	0.03 M Mg
[ ] =	+	+
No Box =	−	+
( ) =	not tested	

Relative template activities of trinucleotides in reactions containing 0.01 or 0.03 M Mg^{++}. A plus (+) sign in the legend means that the trinucleotide stimulates AA-sRNA binding to ribosomes at that magnesium concentration; a minus (−) sign means it is relatively inactive as a template. The results refer to AA-sRNA from *E. coli* strains B and/or W3100. The data are from Anderson, Nirenberg, Marshall, and Caskey (1966).

by T2 bacteriophage results, within one to three minutes, in the modification of one or more species of Leu-sRNA present in the *E. coli* host. Concomitantly, *E. coli*, but not viral protein synthesis is inhibited. Protein synthesis is required, however, for modification of Leu-sRNA.

In collaboration with N. and T. Sueoka, modification of Leu-sRNA has been correlated with codon recognition specificity. sRNA preparations were isolated from *E. coli* before phage infection and at one and eight min after infection. After acylation, Leu-sRNA preparations were purified by MAK column chromatography and the binding of each pooled Leu-sRNA fraction to ribosomes in response to templates was determined (Fig. 6). The profile of Leu-sRNA (eight min after infection) acylated with yeast, rather than *E. coli*. Leu-sRNA synthetase is shown also (Fig. 6D); thus, both anticodon and enzyme recognition sites were monitored. In Fig. 7 the approximate chromatographic mobility on MAK columns of each Leu-sRNA fraction is shown diagrammatically, together with the relative response of each fraction to tri- and polynucleotide templates and acylation specificity of *E. coli* and yeast Leu-sRNA synthetase preparations.

Within one minute after infection, a marked decrease was observed in Leu-$sRNA_2$, responding to CUG, and a corresponding increase was seen in Leu-$sRNA_1$, responding to poly UG, but not to the trinucleotides, UUU, UUG, UGU, GUU, UGG, GUG, GGU, CUU, CUC, CUG, UAA, UAG, UGA, or to poly U or poly UC. However, Leu-$sRNA_1$ was not detected 8 min. after infection.

A marked increase in the response of Leu-$sRNA_5$ to UUG was observed one minute after infection, and an even greater increase was seen eight minutes after infection.

Greater responses of Leu-$sRNA_3$ and Leu-$sRNA_{4a,b}$ to poly UC also were observed eight minutes after phage infection. Leu-sRNA fractions 3 and 4 differ in chromatographic mobility and in acylation specificity by yeast and *E. coli* Leu-sRNA synthetase preparations. Thus, Leu-$sRNA_3$ and a component in fraction 4 differ, although both fractions 3 and 4 respond to poly UC. The multiple responses of Leu-$sRNA_{4a,b}$ to poly U, poly UC, and the trinucleotides, CUU and CUC, suggest that fraction 4 may contain two or more Leu-sRNA species. Striking increases in response of fraction 4 to poly U were observed one and eight minutes after infection.

Leu-sRNA fractions 1, 2, and 3 are related, for each is recognized by yeast as well as by *E. coli* Leu-sRNA synthetase preparations. In contrast, Leu-$sRNA_{4a,b}$ and Leu-$sRNA_5$ are recognized by *E. coli*, but not yeast Leu-sRNA synthetase; thus, fraction 4 is related to fraction 5. Two *different* cistrons of Leu-sRNA are predicted: Leu-sRNA fractions 1, 2, and 3 may be products of one cistron; whereas, fractions 4 and 5 may be products of a different cistron. In this regard, Berg, Lagerkvist, and Dieckman (1962) have shown that *E. coli* Leu-sRNA contains two base sequences at the 4th, 5th, and 6th base positions from the 3′-terminus of the sRNA.

The data suggest the following sRNA precursor-product relationships. Leu-$sRNA_2$ is a product of "cistron A"; the decrease in Leu-$sRNA_2$ and the simultaneous increase in Leu-$sRNA_1$ (within one minute after infection) suggests that Leu-$sRNA_2$ is the precursor of Leu-$sRNA_1$. The data also suggest that Leu-$sRNA_2$ is a precursor of Leu-$sRNA_3$. The following anticodons and mRNA

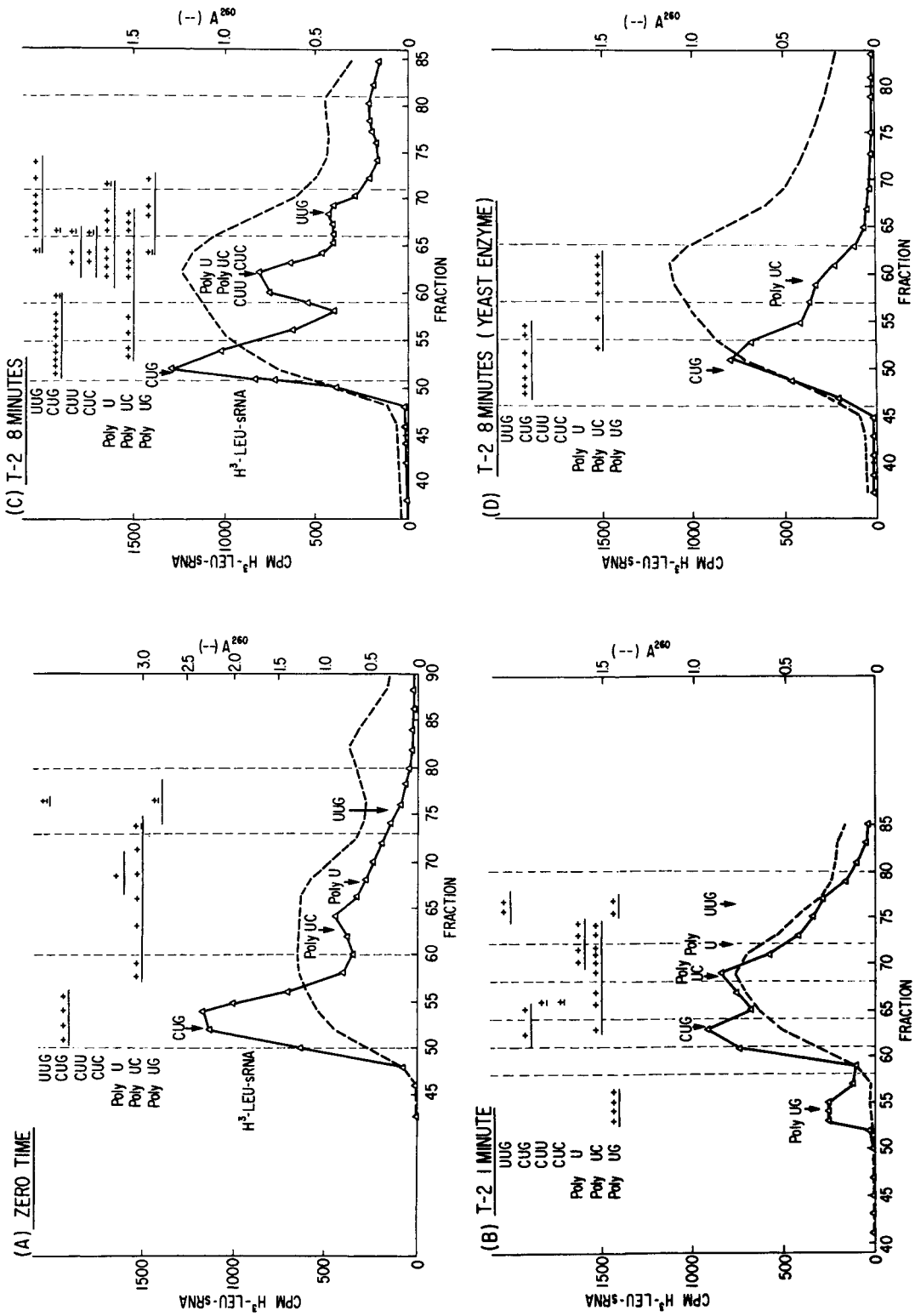
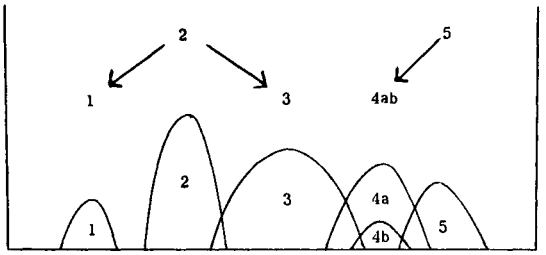


FIGURE 6. The graphs represent MAK column fractions of H^3-Leu-sRNA from *E. coli* B before infection (a) and at 1 min (b) and 8 min (c and d) after infection with T2 phage. sRNA was acylated prior to chromatography with H^3-leucine using *E. coli* (a, b, c) or yeast (d) synthetase preparations. Column eluates were pooled as indicated by the vertical broken lines; dialyzed against 5×10^{-4} M potassium cacodylate, pH 5.5, and lyophilized. Then binding of each fraction to ribosomes in response to tri- or polynucleotide templates was determined. At the top of each graph relative responses of Leu-sRNA fractions to templates are shown. Approximate relative responses are indicated as follows: No symbol, no detectable response of Leu-sRNA; ±, possible response; +, slight response; and ++ to +++++, moderate to strong responses. Profiles represented by broken lines indicate A^{260} units; Δ–Δ, represent H^3-Leu-sRNA. Data are from Kano-Sueoka, Nirenberg, and Sueoka (unpubl.). Also see Sueoka et al., this volume.



CODON	(-)UG POLY UG	CUG	CU(-) POLY CU	UU(-) CUU CUC POLY U POLY CU	UUG POLY UG
ZERO TIME	±	++++	++	+	+
T-2 1"	++	++	++	+	++
T-2 8"	±	+++	+++	+++	+++
AA-sRNA SYNTHETASE					
YEAST	+	+	+	—	—
E.COLI	+	+	+	+	+

FIGURE 7. Diagrammatic representation of the data shown in Fig. 6. The relative mobilities of multiple species of Leu-sRNA, before and after phage infection, fractionated by MAK column chromatography, are shown at the top. Leu-sRNA peaks are numbered. Arrows represent predicted Leu-sRNA precursor-product relationships (Fractions 2 and 5 possibly are products of different cistrons).

Tri- and polynucleotide codons recognized by each Leu-sRNA peak are shown below. Approximate relative responses of Leu-sRNA$_{1\text{-}5}$ to codons are indicated as follows: ±, possible response, + to ++++, slight to strong responses.

On the bottom are shown the specificities of *E. coli* (zero time, 1 and 8 min after infection) and yeast (8 min after infection only) Leu-sRNA synthetase preparations for sRNA^{Leu} fractions.

codons are suggested for Leu-sRNA fractions 2, 3, and 1, respectively (note: asterisks represent modifications of a nucleotide base; codon and anticodon sequences are written with 3′,5′-phosphodiester linkages; antiparallel hydrogen bonding between codon and anticodons is assumed): Leu-sRNA$_2$-product of "cistron A", CAG anticodon, [CUG codon]; Leu-sRNA$_3$- derived from fraction 2, C*AG anticodon, [CU(—) codon]; Leu-sRNA$_1$- derived from fraction 2, CAG** anticodon, [(—)UG codon].

Leu-sRNA$_5$ is a product of "cistron B", and differs from Leu-sRNA$_2$ in anticodon and Leu-sRNA synthetase recognition sites. The sequence, CAA, is suggested for the Leu-sRNA$_5$ anticodon, corresponding to a UUG mRNA codon. Leu-sRNA$_{4a,b}$ are derived from fraction 5. Possible anticodons and codons are: C*AA anticodon, [UU(—) codon]; C*IA anticodon, [UU(—), UC(—), UA(—) codons]; C*AI anticodon, [UU(—), CU(—), AU(—) codons].

Since modification of Leu-sRNA after phage infection is dependent upon protein synthesis, enzyme(s) may be needed to modify bases in Leu-sRNA fractions.

The inhibition of host *E. coli*, but not viral protein synthesis following viral infection may result from modification of Leu-sRNA fractions. N-formyl-Met-sRNA$_1$ serves as an initiator of protein synthesis in *E. coli* and responds to two trinucleotides, UUG and CUG, which are also recognized by Leu-sRNA fractions (see previous discussion on special function codons). Possibly, initiation or termination of *E. coli*, but not viral protein synthesis is affected. Further studies are needed, however, to elucidate the mechanism of viral induced inhibition of host protein synthesis.

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