RNA CODEWORDS AND PROTEIN SYNTHESIS, VIII. NUCLEOTIDE SEQUENCES OF SYNONYM CODONS FOR ARGININE, VALINE, CYSTEINE, AND ALANINE

BY R. BRIMACOMBE, J. TRUPIN,* M. NIRENBERG, P. LEDER, M. BERNFIELD, AND T. JAOUNI

NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

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By directing the formation of AA-sRNA-ribosome complexes with trinucleotide templates, base sequences of RNA codons have been determined. (For brevity, trinucleoside diphosphates are referred to as trinucleotides.) Thus far, the template activities of 45 trinucleotides have been reported.[1-6] In this paper 11 additional trinucleotide sequences are described. CpGpU and CpGpG served as RNA codons for arginine, GpCpC, GpCpA, and GpCpG corresponded to alanine, UpGpC[6] to cysteine, and GpUpC, GpUpA, and GpUpG to valine. ApUpA, ApGpG, and CpUpA had little detectable template activity for C^{14}-AA-sRNA. Similar results have been obtained by Khorana and his co-workers,[7, 21] and, in the case of ApUpA, by Thach, Sundararajan, and Doty.[8]

Materials and Methods.—Components of reactions: The preparation of *E. coli* W3100 ribosomes,[9] sRNA,[10] components of reaction mixtures, and assay for ribosomal-bound sRNA[1] have been described. Each sRNA preparation was acylated with one H^3- or C^{14}-amino acid and 19 C^{12}-amino acids. The properties of the radioactive AA-sRNA preparations not described previously[5, 6] are shown in Table 1. The purity of the Thr-, Cys-, and Ser-sRNA preparations was checked by deacylating the sRNA in ammonium formate (0.1 *M*, pH 10) for 1 hr at 37°, fol-

TABLE 1

RADIOACTIVE AMINOACYL-sRNA PREPARATIONS*

Radioactive amino acid†	Specific radioactivity μcuries/μmole	C^{14}- or H^3-AA-sRNA Added to Each Reaction: A^{260} units	$\mu\mu$moles of C^{14}- or H^3-amino acid accepted	Origin of sRNA *E. coli* strain‡
Ala	88	0.63	16.9	W3100
Arg	193	0.49	14.1	W3100
Expt. *b*	193	0.49	13.7	W3100
Cys-H^3	500	0.75	8.1	B
$-H^3$ Expt. *b*	500	0.75	17.0	B
Glu	205	0.59	11.1	B
His	240	0.38	3.4	B
Expt. *b*	220	0.53	11.5	B
Leu	222	0.12	10.5	B
Expt. *b*	160	0.15	18.4	B
Lys	209	0.30	11.8	B
Met	149	0.39	14.2	W3100
Phe-C^{14}	333	0.49	7.8	W3100
$-H^3$ Expt. *b*	4320	0.09	4.2	B
Pro	158	0.58	13.6	W3100
Ser	120	0.42	13.1	B
Expt. *b*	96	0.60	23.1	B
Thr	160	0.34	14.7	B

* Other AA-sRNA data have been described.[5, 6]

† Amino acids stated were labeled with C^{14} with the exception of H^3-phenylalanine (expt. *b*), and H^3-cysteine (expts. *a* and *b*).

‡ *E. coli* W3100 is a K12 strain. Aminoacyl-sRNA synthetase preparations were from *E. coli* W3100. We thank Dr. Charles Yanofsky for the *E. coli* A-23 strain, and Dr. Ray Byrne for the H^3-Gly-sRNA.[6] A-23 sRNA and 100,000 × *g* supernatant fractions were used for the preparation of H^3-Gly-sRNA.

lowed by electrophoresis in 4% formic acid to separate the free amino acids; a 5% contamination could be detected under the conditions employed.

Synthesis and characterization of oligonucleotides: CpU, GpC, and GpU were obtained from Gallard-Schlessinger Corp., and were purified by chromatography on DEAE-cellulose prior to use. CpG was obtained as a contaminant of the GpC. Other dinucleoside monophosphate preparations have been described.[6]

Primer-requiring polynucleotide phosphorylase was used to catalyze the synthesis of ten trinucleotides (Table 2) from appropriate dinucleoside monophosphates and nucleoside 5'-pyrophosphates[11] (cf. ref. 12). The synthesis of CpGpU, from cytidine 2',3'-cyclic phosphate and GpU, was catalyzed by a derivative of bovine pancreatic ribonuclease.[4, 13] Each trinucleotide was purified by paper chromatography and electrophoresis.[1-4, 11] Prior to chromatography, oligonucleotide solutions rich in G were placed in a water bath at 70° for a few minutes to reduce G-G interactions. The purity, chain length, and base sequence of each trinucleotide preparation were assessed as previously described[1-4, 11] (Table 2).

Results and Discussion.—In Table 3 are shown the effects of 11 trinucleotides upon the binding to ribosomes of 20 AA-sRNA preparations, each acylated with a different C^{14}- or H^3-amino acid. In addition, near the bottom of the table are shown the effects of 45 trinucleotides, previously described,[1-6] upon appropriate AA-sRNA preparations. Trinucleotide template specificity has been determined in this and our previous studies[1-6, 14] in reactions containing 0.03 *M* Mg^{++}, so that small responses could be detected during the initial survey of the 64 trinucleotides with 20 AA-sRNA preparations. Positive responses of AA-sRNA detected under these conditions are currently being studied at different concentrations of Mg^{++} and under other conditions. As discussed previously,[6] partial or imperfect recognition of a trinucleotide sometimes may suffice for the formation of sRNA-codon-ribosome complexes. Possible examples can be seen in Table 3.

The binding to ribosomes of C^{14}-Ala-sRNA was stimulated markedly by GpCpA and GpCpG, less well by GpCpU,[6] and only slightly by GpCpC. Experiments which will be described elsewhere show that yeast C^{14}-Ala-sRNA also responds to the same codon set, but that the relative response of yeast Ala-sRNA to each

TABLE 2

CHARACTERIZATION OF TRINUCLEOTIDES

Compound	Digestion (T2 ribonuclease) Products	Digestion (T2 ribonuclease) Base ratio	Digestion (Venom phosphodiesterase) Products	Digestion (Venom phosphodiesterase) Base ratio
ApGpG[a]	Ap, Gp, G	1.00/0.95/1.00	A, pG[b]	1.10/2.00
ApUpA	Ap, Up, A	1.00/0.90/1.00	A, pU, pA	1.00/0.90/1.00
CpGpG	Cp, Gp, G[c]	0.95/1.00/1.00	C, pG[c]	0.85/2.00
CpGpU[d]	Cp, Gp, U	1.10/1.00/0.95	C, pG, pU	0.95/1.00/1.00
CpUpA	Cp, Up, A	1.10/0.90/1.00	C, pU, pA	1.00/1.00/1.05
GpCpA	Gp, Cp, A[c]	1.05/0.95/1.00	G, pC, pA	1.05/0.95/1.00
GpCpC	Gp, Cp, C	1.05/0.95/1.00	G, pC	1.15/2.00
GpCpG	Gp, Cp, G	1.00/0.95/1.15	G, pC, pG	1.00/0.85/1.00
GpUpA[a]	Gp, Up, A[c]	1.05/0.90/1.00	G, pU, pA[c]	1.00/0.95/1.00
GpUpC	Gp, Up, C[e]	1.05/1.00/0.90	G, pU, pC	1.05/0.95/1.00
GpUpG	Gp, Up, G[e]	1.05/1.00/0.95	G, pU, pG	1.10/0.90/1.00

[a] Trinucleotide contained a small amount (2–5%) of an unidentified contaminant. The chromatographic mobility of the contaminant in solvent systems A and B[6] precluded the possibility of its being an oligonucleotide of longer chain length.

[b] The digestion mixture contained 4% of guanosine; this presumably arose from slight monoesterase activity in the venom phosphodiesterase, since the ApG used in the synthesis was known to be entirely free from GpA.

[c] The digestion mixture contained another component, 1–2% of the total nucleotide content; the electrophoretic mobility and UV spectrum of this component corresponded in each case to unreacted or partially digested oligonucleotide.

[d] CpGpU was prepared from GpU, using a derivative of bovine pancreatic ribonuclease.

[e] The products of digestion were separated by electrophoresis at pH 2.7 instead of pH 3.5,[11] since the Up and Gp did not completely separate at the higher pH. An electrophoretogram at pH 3.5 showed no unexpected digestion products.

TABLE

TEMPLATE SPECIFICITY OF TRINUCLEOTIDES

	$\Delta\mu\mu$Moles of C^{14}- or H^3-Aminoacyl-sRNA Bound to								
Trinucleotide	C^{14}-Ala	C^{14}-Arg	C^{14}-Asp	C^{14}-Asp-NH_2	H^3-Cys	C^{14}-Glu	C^{14}-Glu-NH_2	H^3-Gly	C^{14}-His
CpGpU	−0.13	**1.73**	−0.04	−0.17	−0.28[b]	−0.04	−0.12	0.11	−0.10[b]
CpGpG	−0.03	**1.49**	0.01	−0.05	−0.11	0.02	−0.27	0.02	−0.07
GpUpC	−0.06	0.01[b]	0.04	0.05	−0.02	−0.01	−0.11	−0.07	0.02
GpUpA	−0.09	−0.01[b]	−0.01	0.02	−0.08	0.04	0.09	0.06	0.00
GpUpG	−0.05	−0.16	0.01	0.02	−0.11	−0.03	−0.12	−0.08	−0.18[b]
GpCpC	**0.18**	−0.03[b]	0.02	−0.03	−0.01	0.00	−0.11	−0.06	−0.01
GpCpA	**2.42**	0.07[b]	0.01	−0.03	−0.02	0.06	0.05	−0.35	0.01
GpCpG	**3.51**	−0.02[b]	−0.01	−0.03	−0.05	0.00	−0.27	−0.11	0.05[b]
CpUpA	−0.03	−0.14	0.01	−0.03	0.02	0.00	−0.03	−0.66	−0.07[b]
ApUpA	−0.04	0.02	0.01	0.00	0.04[b]	−0.02	0.00	−0.15[b]	0.02[b]
ApGpG	−0.04	0.01	0.01	0.05	0.01	0.04	0.09	0.01	−0.03
Minus trinucleotide ($\mu\mu$moles)*	0.50	1.27 0.48[b]	0.18	0.22	0.57 1.00[b]	0.12	1.44	2.41 1.10[b]	0.18 0.67[b]
Trinucleotides previously described ($\Delta\mu\mu$moles)	**1.07** GpCpU	**1.31** CpGpC **1.54** CpGpA **0.10** ApGpA	**1.29** GpApU **1.32** GpApC	**1.19** ApApU **1.50** ApApC	**0.93** UpGpU **0.74** UpGpC‡ **0.10** UpGpA‡ **0.39** ApGpU‡ **0.53** ApGpC‡	**0.62** GpApA	**2.05** CpApA **2.60** CpApG	**3.06** GpGpU **0.78** CpApC	**0.88** CpApU	

* Background binding of C^{14}-aminoacyl-sRNA to ribosomes in the absence of trinucleotides is expressed in $\mu\mu$moles (shown near the bottom of the table). All other values ($\Delta\mu\mu$moles) were obtained by subtracting background binding of C^{14}-AA-sRNA from binding obtained upon addition of a trinucleotide preparation. Different experiments with a particular AA-sRNA are indicated by superscripts *b* or *c*. Reproducible stimulations of AA-sRNA binding due to the addition of trinucleotides are in boldface type. For comparison, the template activities of trinucleotides previously described[1-6] are shown at the bottom of the table. Reactions contained the com-

synonym codon differs from that found with *E. coli* C^{14}-Ala-sRNA.[15] Table 3 shows that the binding of C^{14}-Thr-sRNA was also stimulated by GpCpG, and slightly by GpCpA and GpCpC. However, the C^{14}-Thr-sRNA preparation was found to be contaminated to a small extent with C^{14}-Ala-sRNA (see *Methods*), presumably formed during the acylation reaction. This contamination could account for the anomalous response of Thr-sRNA to these trinucleotides. C^{14}-Arg-sRNA responded to CpGpU and CpGpG, and, as reported elsewhere, to CpGpC, CpGpA, and slightly to ApGpA.[6]

As reported previously, Cys-sRNA responds to UpGpU, but not to GpUpU, UpUpG, UpUpU, ApApA, or CpCpC.[1, 3] We have now determined the response of Cys-sRNA to the trinucleotides previously described[4-6] (which were tested with other AA-sRNA preparations but not with Cys-sRNA), and to the trinucleotides shown in Table 3. H^3-Cys-sRNA responded to UpGpU, UpGpC, ApGpU, ApGpC, and, very slightly, to UpGpA, but not to other triplet sequences. UpGpA had little template activity for any other AA-sRNA,[6] and further work will be necessary before a function can be assigned to this trinucleotide. ApGpU and ApGpC previously were found to stimulate binding to ribosomes of C^{14}-Ser-sRNA, and these sequences were tentatively suggested as codons for serine.[6] However, both trinucleotides are more active templates for H^3-Cys-sRNA than for C^{14}-Ser-sRNA. We have been unable to detect any contamination of the H^3-Cys-sRNA preparation with C^{14}-Ser-sRNA, or vice versa (see *Methods*). Further, we have been unable to detect binding to ribosomes of C^{14}-Ser-sRNA in response to the Cys-codons UpGpU and UpGpC, or of H^3-Cys-sRNA in response to the Ser-codons UpCpU, UpCpC, and UpCpG. The higher template activity of ApGpU and ApGpC for H^3-Cys-sRNA compared to C^{14}-Ser-sRNA suggests that both sequences

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FOR C^{14} - OR H^3-AMINOACYL-sRNA

Ribosomes Due to Addition of Trinucleotide*										
C^{14}-Ileu	C^{14}-Leu	C^{14}-Lys	C^{14}-Met	C^{14}- or H^3-Phe	C^{14}-Pro	C^{14}-Ser	C^{14}-Thr†	H^3-Trypt	C^{14}-Tyr	C^{14}-Val
−0.08	−0.32	−0.10	−0.22	−0.49[b]	−0.08	−0.45[b]	−0.13	−0.05	−0.02	−0.01
−0.05	−0.03	−0.01	−0.03	−0.07	0.08	−0.03	−0.02[b]	−0.01	0.01	0.00[b]
−0.03	−0.16	0.08	−0.01	−0.06	0.01	−0.09	0.03[b]	−0.03	−0.02	**0.75**
−0.05	−0.22	0.08	−0.08	−0.11	−0.06	−0.14	−0.03[b]	−0.03	−0.01	**1.33**
−0.06	−0.20	−0.13	0.10	−0.35[b]	−0.01	−0.38[b]	−0.09	−0.04	0.00	**1.08**
−0.04	−0.16	0.03	−0.05	−0.02	−0.08	−0.15	0.15[c]†	−0.07	−0.01	−0.01[b]
−0.04	−0.26	−0.02	−0.15	−0.21	−0.20	−0.19	0.14[c]†	−0.04	0.00	−0.01[b]
−0.05	−0.32[b]	−0.13	0.00	−0.08	−0.21	−0.05	**0.47**[c]†	−0.06	0.01	0.02[b]
−0.02	**0.05**	**0.25**	−0.10	−0.18[b]	−0.06	−0.14[b]	−0.04	...	0.02	−0.01
0.00	−0.01	0.09	0.07	−0.02[b]	−0.02	0.07[b]	−0.04	0.02	0.01	−0.01
0.00	−0.04	0.01	0.03	−0.04	−0.04	−0.02	−0.01[b]	−0.03	0.01	0.00[b]
0.15	0.91	0.91	0.47	0.48	0.63	1.17	0.63	0.23	0.23	0.30
...	1.44[b]	...	...	1.09[b]	...	2.20[b]	0.28[b]	...	...	0.13[b]
...	...	...	...	...	...	...	0.43[c]	...	...	...
0.64	**0.39**	**2.11**	**1.00**	**1.29**	**0.15**	**1.27**	**0.91**[c]	**0.12**	**0.81**	**1.00**
ApUpU	UpUpG	ApApA	ApUpG	UpUpU	CpCpU	UpCpU	ApCpU	Poly UG	UpApU	GpUpU
0.73	**0.18**	**1.37**	...	**1.59**	**0.08**	**0.83**	**0.50**	...	**0.56**	...
ApUpC	CpUpU	ApApG	...	UpUpC	CpCpC	UpCpC	ApCpC	...	UpApC	...
...	**0.16**	...	...	...	**0.40**	**0.82**	**0.45**	...	...	...
...	CpUpC	...	...	...	CpCpA	UpCpG	ApCpA	...	...	...
...	**0.62**	...	...	...	...	**0.14**	**1.10**[c]	...	...	...
...	CpUpG	...	...	...	...	ApGpU	ApCpG	...	...	...
...	...	...	...	...	...	**0.11**	...	...	...	...
...	...	...	...	...	...	ApGpC	...	...	...	...

ponents described under *Materials and Methods*, the amount of C^{14}-AA-sRNA stated previously[5, 6] or in Table 1, and 0.150 A^{260} units of trinucleotide, as specified, in a final volume of 50 μl. C^{14}-Asp-NH_2-sRNA was assayed in 100 μl reactions; amounts of all components were doubled.

† C^{14}-Thr-sRNA contained some C^{14}-Ala-sRNA.

‡ These trinucleotides were not previously tested with Cys-sRNA.[6]

may serve as Cys-codons. However, since both Cys- and Ser-sRNA consistently respond to ApGpU and ApGpC, further work will be necessary to clarify the status of these trinucleotides.

GpUpC, GpUpA, and GpUpG (cf. ref. 7), as well as GpUpU,[2] served as RNA codons for valine. CpUpA slightly stimulated the binding of C^{14}-Leu- and C^{14}-Lys-sRNA, but C^{14}-Lys-sRNA did not respond to CpUpA in reactions containing 0.01 *M* Mg^{++}. CpUpA, CpUpU, and CpUpC may serve as internal codons for leucine;[4, 7, 16, 17] however, the status of CpUpA remains uncertain. ApUpA and ApGpG had little template activity for any AA-sRNA.

In Table 4 are summarized the nucleotide sequences of the 56 RNA codons reported thus far,[1–6] together with predicted functions of the remaining eight sequences. The results of preliminary experiments, to be reported elsewhere,[18] validate many of these predictions. For example, GpApG, CpCpG, UpGpG, and GpGpC were found to serve as codons for glutamic acid, proline, tryptophan, and glycine, respectively. It should be emphasized that Table 4 does not represent an invariant dictionary, for codon recognition is subject to modification. Further, the relative template activities of synonym codons may vary considerably.

The striking patterns of synonym codon sets have been commented on previously.[5, 6] Recognition of the 3′-terminal base in a trinucleotide is most variable. In every case, each member of a trinucleotide pair with 3′-terminal pyrimidines, such as XpYpU and XpYpC, corresponds to the same amino acid. Codon pairs with 3′-terminal purines, such as XpYpA and XpYpG, often correspond to the same amino acid, but the lack of template activity of ApUpA for Met-sRNA and of CpUpA for Leu-sRNA demonstrates that in some cases there is a large difference in template activity between the codons in such pairs. This raises the possibility that

TABLE 4

NUCLEOTIDE SEQUENCES OF RNA CODONS

UpUpU, UpUpC } Phe	UpCpU, UpCpC } Ser	UpApU, UpApC } Tyr	UpGpU, UpGpC } Cys
UpUpA (?-Leu, Nons)	UpCpA $Ser^{Pred.}$	UpApA } (?-Term)	UpGpA (?-Cys, Trypt, Term)
UpUpG Leu	UpCpG Ser	UpApG }	UpGpG $Trypt^{Pred.}$
CpUpU, CpUpC } $Leu^{Int.}$	CpCpU, CpCpC } $Pro^{Int.}$	CpApU, CpApC } His	CpGpU, CpGpC } Arg
CpUpA (?-Leu, Nons)	CpCpA Pro	CpApA } Glu-NH_2	CpGpA } Arg
CpUpG Leu	CpCpG $Pro^{Pred.}$	CpApG }	CpGpG }
ApUpU, ApUpC } Ileu	ApCpU, ApCpC } Thr	ApApU, ApApC } Asp-NH_2	ApGpU, ApGpC } (?-Cys, Ser, Term)
ApUpA (?-Met, Nons)	ApCpA } Thr	ApApA } Lys	ApGpA } (?-Arg, Term)
ApUpG Met	ApCpG }	ApApG }	ApGpG }
GpUpU, GpUpC } Val	GpCpU Ala	GpApU, GpApC } Asp	GpGpU Gly
	GpCpC $Ala^{Int.}$		GpGpC $Gly^{Pred.}$
GpUpA, GpUpG } Val	GpCpA, GpCpG } Ala	GpApA Glu	GpGpA, GpGpG } $Gly^{Pred.}$
		GpApG $Glu^{Pred.}$	

Summary and predictions: The template activities of all trinucleotides not underlined have been studied experimentally. Preliminary experiments with underlined trinucleotides (except GpGpA and GpGpG) have also been performed. Estimates of relative template efficiencies of synonym codons are not indicated.

The following notation has been used: (?-. . .), assignment uncertain; Term, possible terminator codon; Nons, possible nonsense or special function codon; $AA^{Int.}$, possible internal codon, although little template activity was detected with the trinucleotide; $AA^{Pred.}$, codon assignment predicted; XpYpZ, trinucleotide not studied experimentally.

each member of the pair may have a unique function. A third class of synonym codons in which 3 or 4 bases may serve at the 3′-terminal position in a trinucleotide has also been found. In the case of Leu-codons, pyrimidines are equivalent at the 5′-terminal position.

Amino acids which are structurally or metabolically related, perhaps synthesized from a common precursor, often have similar RNA codons.[6, 16] Since specific rather than random errors are found during polynucleotide-directed amino acid incorporation into protein *in vitro*, a correspondence between amino acid structure and RNA codon structure may provide a mechanism for minimizing the effects of errors during protein synthesis.

Nonsense codons and uncertainties: The term "nonsense" was originally used to denote a nonreadable codon.[19, 20] However, it should be emphasized that each triplet may occur in three structural forms, as a 5′-terminal-, 3′-terminal-, and internal-codon (trinucleotides represent a fourth form), and one form may differ in template activity from another. Also, the assay may not detect codon recognition by nonacylated sRNA, by sRNA acylated with an unlabeled molecule (not one of the 20 amino acids), by an sRNA species present in low concentration, or by sRNA forming an unstable sRNA-ribosome-codon complex.

Fourteen trinucleotides have little detectable template activity for AA-sRNA. Some sequences may serve as readable internal codons, but may be poorly recognized at terminal positions. Probable examples are CpCpU and CpCpC for proline,[4, 6] and CpUpU and CpUpC for leucine, as already mentioned. Other possible examples are GpCpC for alanine, ApGpA for arginine,[6] and CpUpA for leucine. The uncertainties related to ApGpU and ApGpC have been discussed above. On the basis of genetic evidence,[22–24] together with sRNA binding studies,[6] UpApA and

UpApG have been suggested as possible terminator codons. ApGpG, ApUpA, and UpGpA may also be sequences to consider in this context. In a recent study of great interest, Marcker and Sanger[25] have demonstrated that methionine amino groups of *E. coli* Met-sRNA are susceptible to enzymatic formylation (also cf. ref. 26). Thus far, ApUpG is the only trinucleotide sequence which has been found to serve as a codon for methionine.[6] Since a large percentage of *E. coli* protein contains methionine at the N-terminus,[27, 28] ApUpA perhaps is of interest as a possible codon specifying a methionine derivative at N-terminal positions of proteins.

Summary.—Eleven trinucleotides were used as templates to direct the binding of C^{14}-AA-sRNA to ribosomes. The results suggest that CpGpU and CpGpG serve as RNA codons for arginine, GpUpC, GpUpA, and GpUpG for valine, GpCpC, GpCpA, and GpCpG for alanine, and UpGpC[6] for cysteine. CpUpA, ApUpA, and ApGpG had little detectable template activity for AA-sRNA. Possible nonsense codon sequences are discussed.

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The following abbreviations are used: AA-sRNA, aminoacyl-sRNA; Ala-, alanine; Arg-arginine; Asp-NH_2-, asparagine; Asp-, aspartic acid; Cys-, cysteine; Glu-, glutamic acid; Glu-NH_2-, glutamine; Gly-, glycine; His-, histidine; Ileu-, isoleucine; Leu-, leucine; Lys-, lysine; Met-, methionine; Phe-, phenylalanine; Pro-, proline; Ser-, serine; Thr-, threonine; Tryp-, tryptophan; Tyr-, tyrosine; Val-, valine; sRNA, transfer RNA; U, uridine; C, cytidine; A, adenosine; G, guanosine.

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*HISTONE-BOUND RNA, A COMPONENT OF NATIVE NUCLEOHISTONE**

BY RU-CHIH C. HUANG AND JAMES BONNER

DIVISION OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

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We have found that native histone molecules as they occur in the nucleohistone component of pea bud chromatin contain RNA molecules chemically linked to them. The histone-bound RNA constitutes a new class of RNA, differing from other classes in base composition and in chain length. That the RNA content of native histone has not been discovered heretofore is due to the fact that histones are commonly extracted from chromatin with acid, a condition under which the RNA-histone bond is cleaved. Since the nucleohistone component of chromatin is inactive in the support of DNA-dependent RNA synthesis and since the histones are one class of agents responsible for such repression, their association with a specific class of RNA is relevant to considerations of the control of genetic activity.

Materials and Methods.—P^{32}-labeled nucleohistone: We have used P^{32}-labeled histone-RNA complex for the major part of this work. For each preparation *ca.* 5 kg of pea seeds (var. Alaska) were soaked in such a volume of P^{32}-solution (50 mc carrier-free $H_3P^{32}O_4$ adjusted to pH 7.5 with 1 *M* tris) that all was imbibed by the seeds. These were then planted in vermiculite and germinated in the dark for 6 days at 25°C. The apical buds were then removed, each preparation yielding *ca.* 600 gm fresh weight of buds. From these, chromatin was prepared by the methods of Huang and Bonner.[1] The buds were ground (Waring Blendor) in grinding medium consisting of sucrose 0.25 *M*, tris buffer, pH 8, 0.05 *M*, and $MgCl_2$, 0.001 *M*, the homogenate was filtered successively through cheesecloth and Miracloth, and pelleted for 30 min at 4000 × *g*. The crude chromatin was scraped from the underlying starch, resuspended in grinding medium, and repelleted for 10 min at 10,000 × *g*. The crude chromatin was then repelleted 4 further times from 0.05 *M* tris pH 8. The 5 × repelleting of the crude chromatin removes much nonchromosomal protein and contributes importantly to the purity of the final product. The thus purified crude chromatin was next layered on 1.7 *M* sucrose, and the upper third of the tube stirred to form a rough gradient. It was then centrifuged for 105 min at 22 krpm in the SW25 Spinco head. The chromatin pellets as a clear gelatinous material. At this stage we recover *ca.* 600 OD_{260} (approximately 30 mg) of chromosomal DNA. The purified chromatin was next dialyzed for 1 hr against 4 × diluted dilute saline citrate (DSC, 0.015 *M* NaCl, 0.001 *M* Na citrate) and then sheared to produce soluble nucleohistone by the method of Bonner and Huang.[2] The chromatin, at a concentration of 0.5 mg DNA/ml, was sheared for 90 sec in a Waring Blendor at 80 v. The suspension was then centrifuged for 30 min at 10 krpm. The supernatant contains the soluble nucleohistone. Approximately 70% of the chromosomal DNA is recovered as soluble nucleohistone. In general, the soluble nucleohistone was concentrated, as well as freed of contamination by small molecules, by pelleting for 16 hr at 40 krpm in the no. 40 Spinco head.