fects changes the absorbance: (1) Unstacking the nucleotide pairs increases absorbance by approximately 45 per cent at 250–280 mμ. (2) The difference spectrum for reaction of a T2-mimetic mixture of adenine, guanine, and cytosine with CH_2O has its isosbestic points near 255 mμ and maximum at 275 mμ.[14] We estimate that the combined effects of denaturation and formylation lead to fractional absorbance increases, r, of 0.45, 0.49, and 0.54 at 255, 258, and 260 mμ respectively. The absorbance that ordered C-RNA contributes to the solution (A_h) is therefore a^{Δ}/r. f_h is calculated from A_h and A_0, the absorbance before CH_2O addition, as

$$f_h = (1 + r)A_h/(A_0 + A_h) = (1 + r)a^{\Delta}/r(A_0 + a^{\Delta}).$$

For sample 11, the values of f_h calculated in this way at 255, 258, and 260 mμ are 0.57, 0.59, and. 0.55 respectively. Averaged values of f_h are listed in Table 1. They are consistently lower than f_h calculated from the thermal transition data (e.g., Fig. 1).

[14] Haselkorn, R., and P. Doty, *J. Biol. Chem.*, **236**, 2738 (1961).

[15] Geiduschek, E. P., *J. Mol. Biol.*, in press (1962).

[16] It has already been shown that when T2 DNA and T2-C-RNA are heated together to 100°C and quenched, no complex formation can be detected in a CsCl density gradient. Such complexes form during the "annealing" at 41°C.[8, 17] The reheating of T2 DNA together with the C-RNA, therefore, in no way complicates the interpretation of this experiment.

[17] Hall, B. D., and S. Spiegelman, these PROCEEDINGS, **47**, 137 (1961).

[18] Marmur, J., and D. Lane, these PROCEEDINGS, **46**, 451 (1960).

[19] Doty, P., J. Marmur, J. Eigner, and C. Schildkraut, these PROCEEDINGS, **46**, 461 (1960).

[20] Recently, Nakamoto and Weiss, these PROCEEDINGS, **48**, 880 (1962), have shown that the enzyme preparations used for the DNA-primed synthesis of C-RNA also catalyze an RNA-primed RNA polymerization. It is therefore possible that part of the RNA isolated for these experiments is made on a C-RNA rather than a DNA template. However, the relative rates of these two processes are such that under our synthetic conditions not more than 5 per cent of the C-RNA could have been made by the RNA-primed pathway. The self-complementarity of at least 85 per cent of T2-C-RNA (Fig. 6) must therefore be a property of the DNA-primed RNA synthesis.

ON THE ROLE OF SOLUBLE RIBONUCLEIC ACID IN CODING FOR AMINO ACIDS[*,†]

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In protein synthesis, each amino acid is first joined specifically with a corresponding sRNA through the mediation of an activating enzyme. These aminoacyl-sRNA's, by reaction with a ribosomal preparation,[1, 2] form proteins with specific amino acid sequences.[3] According to the "adaptor" hypothesis of Crick[4] and Hoagland,[5] the position of a particular amino acid would be determined not by the amino acid itself, but by hydrogen bonding between the RNA template and a complementary nucleotide sequence in the sRNA carrying the amino acid. The experiment described in this paper was designed as a direct test of the adaptor hypothesis, by attaching an amino acid to its normal sRNA and then, without breaking the bond, converting the amino acid to another one of the natural amino acids. It is then possible to determine whether the coding properties of this hybrid are determined by the sRNA or the amino acid. We have made use of the fact that cysteine can be altered by reductive desulfhydration with Raney Nickel to alanine.

This reaction can be performed while the cysteine is attached to its sRNA, producing the hybrid Ala-sRNA^{CySH}. A superscript denotes the normal amino acid accepting specificity of an sRNA, the actual amino acid attached being indicated as a prefix. Figure 1 illustrates this procedure.

To determine the coding properties of the hybrid molecule, we have utilized the finding by Nirenberg and Matthaei,[6] Lengyel, Speyer, and Ochoa,[7] Speyer, Lengyel, Basilio, and Ochoa,[8] and Martin, Matthaei, Jones, and Nirenberg,[9] that polyuridylic-guanylic acid will stimulate ribosomal incorporation into polypeptides of certain amino acids, including cysteine, but not alanine. As shown below, this difference between cysteine and alanine also applies when they are attached to their normal acceptors, i.e., CySH-sRNA^{CySH} is reactive with poly UG but Ala-sRNA^{Ala} is not. The hybrid molecule Ala-sRNA^{CySH} proved to be just as reactive as CySH-sRNA^{CySH}, leading to the conclusion that the sRNA moiety indeed determines the coding specificity.

Materials and Methods.—Preparation of C^{14}-L-cysteine: L-C^{14}-cysteine of high specific activity was prepared from L-C^{14}-serine. The yeast serine sulfhydrase described by Schlossmann and Lynen[10] catalyzes the reaction:

$$CH_2OH{\cdot}CHNH_2{\cdot}COOH + H_2S \rightarrow CH_2SH{\cdot}CHNH_2{\cdot}COOH + H_2O.$$

The enzyme was prepared from National bakers' yeast which had been frozen in liquid nitrogen and stored at $-20°$; 50 gm of frozen yeast were extracted by stirring at 3° for 5 hr with 100 ml of 0.05 M K_2HPO_4 and 0.05 M EDTA. From then on, the procedure of Schlossmann and Lynen was followed up to the ammonium sulfate step. The ammonium sulfate precipitate between 40 and 65 per cent saturation was dissolved in 15 ml of 0.05 M Tris HCl, pH 7.5, and dialyzed against 0.02 M Tris HCl buffer. The dialysate was stored at $-20°$. 0.75 μmole of C^{14}-L-serine (65 μC/μmole, New England Nuclear Corporation) was dissolved in a total volume of 1.5 ml containing: 3 μmoles of EDTA; 0.5 ml of 0.5 M Tris HCl, pH 8.5, saturated with H_2S; 0.3 μmole of pyridoxal phosphate; and 2 mg of enzyme. After incubation at 37° for 3 hr in nitrogen, 20 ml of ethanol containing 0.2 ml of 2 N HCl were added to the cysteine. After centrifugation, the supernatant was evaporated and the residue re-extracted with 10 ml ethanol plus 0.2 ml 2 N HCl, and the extraction and evaporation were repeated once more.

Before the ethanol addition, 75 μmoles of C^{12}-serine, 20 μmoles of C^{12}-alanine, and 30 μmoles of C^{12}-glycine were added to dilute residual radioactivity of the serine and possible degradation products. The repeated extraction-evaporation procedure was used, since cysteine is more soluble in ethanol than the other amino acids. The final product contained approximately 40 per cent of the input radioactivity. On paper electrophoresis at pH 1.85 in 7.8 per cent acetic acid and 2.5 per cent formic acid (70 volts/cm, 60 min), no detectable radioactivity was found except with cysteine. The dry product was stored at $-20°$.

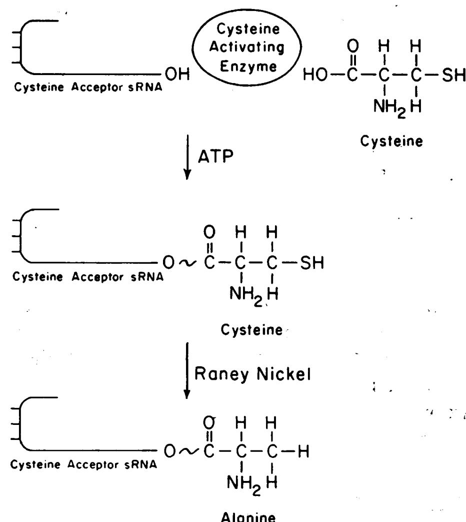
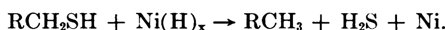


FIG. 1.—Plan of experiment. Cysteine is attached to its normal acceptor sRNA through the mediation of the cysteine activating enzyme. By the action of Raney Nickel, the cysteine, while still attached, is converted to alanine. The coding properties of the hybrid molecule are then investigated.

Preparation of C^{14}-CySH-sRNA^{CySH}: *E. coli*-sRNA was prepared as described,[11] and a 105,000

$\times$ *g* supernatant of *E. coli* extract, passed through a Sephadex G-25 column to remove amino acids, was used for charging. While charging with C^{14}-cysteine, β-mercaptoethanol was added. After incubation and phenol extraction, a 100-fold excess of C^{12}-cysteine was added. The alcohol precipitate of the phenol extract was dissolved in 0.01 *M* mercaptoethanol and dialyzed at 3° for 16 hr against 10^{-4} *M* mercaptoethanol solution. To test the charged sRNA, a sample was electrophorized at pH 1.85 as described above for the identification of cysteine. Radioactivity was detected only in the ultraviolet-absorbing, nonmoving position. However, when the charged sRNA was hydrolyzed, with addition of C^{12}-cysteine, for 30 min at pH 11, the radioactivity now appeared in the pH 1.85 electropherogram in the region common, under these conditions, for both cysteine and cystine. Frequently in this procedure, cysteine yielded some material that moved more slowly, presumably due to oxidation. With different batches of sRNA, variable amounts of C^{14}-cysteine were charged, for example, 4,000 and 7,200 cpm/mg RNA, corresponding to 0.067 and 0.12 mμmole/mg respectively. When C^{14}-phenylalanine was charged on these preparations, a variation of ratios of cpm/mg in the same sense was observed.

Reduction of CySH-sRNA^{CySH} to Ala-sRNA^{CySH}: Raney Nickel No. 28, suspended in water, was obtained from Raney Catalyst Company, Chattanooga, Tennessee, and stored at 0°. The preparation was washed with water before use. Organic sulfur compounds are easily reduced by Raney Nickel.[12, 13] It acts at room temperature, and, in the case of cysteine, noncatalytically (cf. for example, Mozingo *et al.*[13]):



$$RCH_2SH + Ni(H)_x \rightarrow RCH_3 + H_2S + Ni.$$

Generally, 1.5 ml of a solution containing 8.5 mg of C^{14}-CySH-sRNA^{CySH} (53,000 cpm), 0.4 *M* acetate buffer, pH 5, 0.12 ml of saturated EDTA, and 170 mg of Raney Nickel was shaken at room temperature for 30 min. After adding another 0.12 ml of saturated EDTA solution, the Nickel was centrifuged off. To the clear supernatant, 2 vol of cold ethanol were added and the mixture kept at −20° for 2 hr. After centrifugation, the precipitate was taken up in 0.5 ml of water and dialyzed against water overnight at 3°; 18,000 cpm on 5.25 mg. of RNA were nondialyzable, and on paper electrophoresis at pH 1.85 the radioactivity and the ultraviolet-absorbing material were found in the nonmoving portion. After hydrolysis for 30 min at pH 11 and electrophoresis at pH 1.85 in the presence of 20 μg each of C^{12}-cysteine and alanine, the radioactivity moved away from the ultraviolet spot and approximately 70 per cent was found in the region corresponding to alanine and 30 per cent in that of cysteine. Reduction of CySH-sRNA^{CySH} varied under these conditions between 30 and 70 per cent. During the reduction procedure about half the sRNA-bound amino acid was lost.

Preparation of polynucleotide phosphorylase: Polynucleotide phosphorylase was prepared from lyophilized *Micrococcus lysodeikticus* (Miles Chemical Co., Clifton, N.J.) by the method of Singer and Guss.[14]

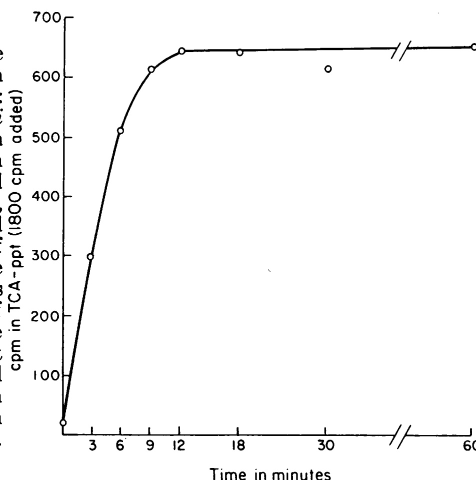
Preparation of poly UG: The reaction mixture contained in a total volume of 1.2 ml: 25 μmoles of UDP and 5 μmoles of GDP neutralized at pH 8.2; 5 μmoles of $MgCl_2$; 1 μmole of EDTA; 100 μmoles of Tris HCl buffer pH 8.2; and approximately 5 units[14] of the enzyme. Incubation was for 85 min at 35°. After addition of phenol as in the preparation of sRNA, the poly UG was isolated, redissolved, dialyzed against water, and lyophilized. Approximately 3 mg of material were obtained; when dissolved in 2 ml, it gave a viscous solution containing 46 optical density units per ml at 260 mμ. The ratio of 280 to 260 absorption was 0.38.

In the experiments with Raney Nickel-treated Phe-sRNA^{Phe}, polyuridylic acid was used that had been prepared as described for poly UG, except that UDP only was added.

E. coli ribosomes were prepared from frozen bacterial paste by the method described.[11] Crude 105,000 $\times$ *g E. coli* supernatant was prepared by centrifugation for 2 hr of 20 gm of alumina-ground cell paste extracted with 60 ml of Tris Mg acetate.

Results.—Speyer *et al.*[8] and Martin *et al.*[9] showed that, in the presence of phenylalanine, poly UG stimulates the ribosomal incorporation of cysteine but not of alanine. It had been shown that such transfer can occur directly from aminoacyl-sRNA, at least in the case of phenylalanine.[15] The time curve in Figure 2 shows the transfer of cysteine from CySH-sRNA^{CySH} in the presence of poly UG. In the absence of poly UG, the incorporation in such an experiment is typically about

FIG. 2.—Time curve of the transfer of cysteine from sRNA into TCA-insoluble product. Each sample contained, in a total volume of 0.25 ml: 2 mg of *E. coli* ribosomes, 0.25 mg of *E. coli* 105,000 × *g* crude supernatant (expressed in mg of protein), 0.4 mg of sRNA charged with C^{12}-phenylalanine, 0.25 mg of sRNA charged with C^{14}-cysteine (1,800 cpm), 0.01 *M* PEP, 8 μg of PEP-kinase, 0.01 *M* GTP, 0.012 *M* reduced glutathione, 0.012 *M* $MgCl_2$, 0.03 *M* KCl, 10 μg of poly UG, and 0.05 *M* Tris HCl pH 7.4. The incubation was at 35° and the reaction was stopped in each tube by the addition of 5 ml of 5 per cent TCA at the indicated time. The precipitate was extracted with this TCA at 90° for 15 min, washed twice with TCA and once with 1:1 cold ether/alcohol. It was then dissolved in formic acid, plated, and counted in the Nuclear Chicago windowless gas flow counter.



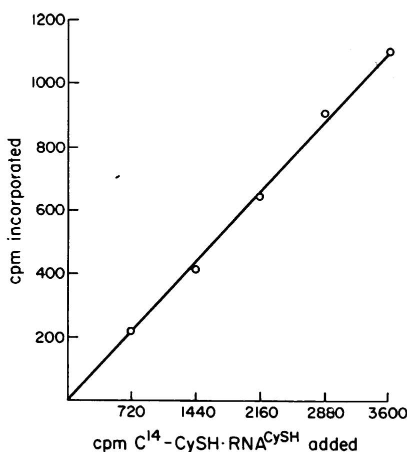


FIG. 3.—Amount of C^{14}-cysteine transferred versus input. Incubation was for 30 min. The conditions were the same as in Figure 2, except that 1.5 mg of ribosomes were used. The specific activity was 7,200 cpm/mg RNA.

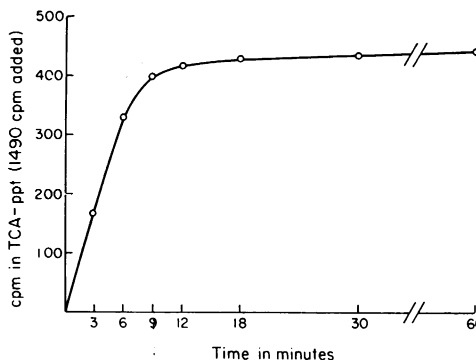


FIG. 4.—Time curve with Raney Nickel-treated C^{14}-CySH-sRNA^{CySH} containing 60 per cent of the radioactivity as alanine and 40 per cent as cysteine. Conditions were the same as in Figure 2, except that 0.5 mg of sRNA (1,490 cpm) was used for each incubation.

200 cpm. As shown in Figure 3, the incorporation with different amounts of CySH-sRNA^{CySH} is proportional to the input, about one third being incorporated. Incorporation in the absence of poly UG was found generally to amount to about 20 per cent of that with poly UG.

When CySH-sRNA^{CySH} was treated with Raney Nickel and about 60 per cent of the radioactivity had been converted to Ala-sRNA^{CySH}, about one third of the total counts were incorporated into the insoluble fraction, as seen in Figure 4, a result comparable to that obtained with the unreduced preparation. The similarity of the time curves for incorporation with reduced and unreduced preparations indicates that exposure to Raney Nickel did not affect the sRNA except that some amino acid was lost. To confirm this further, Phe-sRNA^{Phe} was subjected to a Raney Nickel treatment analogous to that used for the reduction of CySH-

TABLE 1

LACK OF EFFECT OF RANEY NICKEL TREATMENT ON POLY-U-DEPENDENT TRANSFER OF PHENYLALANINE FROM C^{14}-PHE-sRNA^{Phe}

	C^{14}-Phe-sRNA^{Phe}	TCA precipitate Without poly-U	TCA precipitate With poly-U
	(cpm)	(cpm)	(cpm)
Untreated	1,295	108	775
Raney Nickel-treated	1,280	111	782

C^{14}-Phe-sRNA^{Phe} (70 O.D. units at 260 mμ, 18,500 cpm) was treated with Raney Nickel as described for C^{14}-CySH-sRNA^{CySH}. After this treatment, 49.5 O.D. units containing 9,200 cpm were recovered. The C^{14}-Phe-sRNA^{Phe} was incubated with 0.5 μg of poly-U in 0.5 ml under the conditions described in Figure 2.

sRNA^{CySH} and, as shown in Table 1, incorporation of phenylalanine in the presence and absence of poly-U is unchanged by the treatment. It was also found that the acceptor activity of sRNA, at least for arginine, is not damaged by Raney Nickel.

As shown in Table 2, poly UG does not stimulate incorporation with authentic

TABLE 2

POLY UG-STIMULATED INCORPORATION FROM RANEY NICKEL-TREATED C^{14}-CYSH-sRNA^{CySH}

	Aminoacyl-sRNA added: CySH-sRNA^{CySH}	Ala-sRNA^{Ala}	Ala-sRNA^{CySH}	Incorporation into TCA Insoluble Product: Without poly UG	With poly UG
	(cpm)	(cpm)	(cpm)	(cpm)	(cpm)
Untreated	1800	—	—	162	508
Untreated	—	2800	—	158	169
Raney Nickel-treated CySH-sRNA^{CySH}	650	—	840	67	527

Conditions were as in Figure 2, except that the volume of the medium was 0.5 ml and 4 mg of ribosomes were used. Incubation was for 30 min.

Ala-sRNA^{Ala}. Therefore, the result described in Figure 4 indicates that reduction of the cysteine on sRNA to alanine does not alter its incorporation. To prove the incorporation from Ala-sRNA^{CySH} unambiguously, the TCA-extracted residue was hydrolyzed and analyzed. To simplify the analysis, the cysteine was oxidized with performic acid to give cysteic acid. After evaporation, the oxidized hydrolysate was dissolved in 0.1 *N* HCl and run over a small column of Dowex 50; this retains alanine but lets cysteic acid pass through. The alanine was then eluted with 4 *N* HCl. An experiment of this type is presented in Table 3. In this case, large

TABLE 3

ANALYSIS OF OXIDIZED HYDROLYSATE OF TCA-PRECIPITATE SEPARATION ON DOWEX 50

	Input: CySH-sRNA^{CySH} (cpm)	Input: Ala-sRNA^{CySH} (cpm)	Incorporated—Dowex 50 analysis: Total (cpm)	Filtrate Cysteic acid (cpm)	Eluate alanine (cpm)
Untreated CySH-sRNA^{CySH}	9,000	—	3,050	2,835	83
Raney Nickel-treated CySH-sRNA^{CySH}	3,500	6,500	3,290	990	2,020

To the 2 ml reaction mixture, incubated at 37° for 30 min, 5 ml of 7 per cent TCA were added. After heating for 15 min at 90°, the insoluble material was centrifuged, resuspended, and washed 3 times with 5 per cent TCA at room temperature. It was then washed with 5 ml of cold alcohol/ether solution and spun down at 0°. To the final precipitate, 5 μmoles of C^{12}-cysteine, 2.5 μmoles of C^{12}-alanine, 0.1 ml of pure formic acid, and 1.5 ml of 6 *N* HCl were added. The mixture was hydrolyzed in the autoclave in sealed tubes for 28 hr at 105°. The HCl was evaporated in a rotary evaporator, and the dry material dissolved in 0.5 ml of performic acid and heated at 50° for 1 hr. The performic acid was evaporated in an air current at 50°, and the residue taken up with water and put on a Dowex 50 column in the acid form (0.6 × 4.5 cm). The column was washed with 5 ml of 0.1 *N* HCl. The filtrate and acid-washing fluid containing cysteic acid were combined and kept. Alanine and other amino acids were eluted from the column with 4 ml of 6 *N* HCl. Filtrate and eluate were evaporated and redissolved in water, and an aliquot of each used for counting radioactivity and for paper electrophoresis.

TABLE 4

ANALYSIS OF OXIDIZED HYDROLYSATE OF TCA-PRECIPITATE BY ELECTROPHORETIC SEPARATION

	Input		Incorporated-Electrophoretic Analysis		
	CySH-sRNA^{CySH} (cpm)	Ala-sRNA^{CySH} (cpm)	Total (cpm)	Cysteic acid spot (cpm)	Alanine spot (cpm)
Untreated CySH-sRNA^{CySH}	2,870	—	520	225	3
Raney Nickel-treated CySY-sRNA^{CySH}	660	1,540	395	66	141

A small-scale incubation was made, as described in Table 3, except that before hydrolysis only 0.15 μmole of C^{12}-cysteine and 0.15 μmole of C^{12}-alanine were added. The hydrolysate was oxidized with performic acid and electrophorized under the same conditions as in Figure 5. The alanine and cysteic acid spots were eluted and counted.

amounts of incubate were analyzed, and it may be seen that again about one third of the counts present in either the reduced or unreduced input were incorporated into the TCA-insoluble material. With the treated preparation containing 65 per cent of its counts as Ala-sRNA^{CySH}, two thirds of the counts were retained on the column and appeared in the Dowex eluate as expected for alanine.

As a further check, the Dowex filtrates and eluates from the experiment in Table 3 were compared by electrophoresis followed by radioautography. As shown in Figure 5, radioactivity was found in the position corresponding to alanine, whereas no radioactivity appeared in this area with the untreated CySH-sRNA^{CySH}. Cysteic acid moved slightly in the opposite direction and was proportionally weaker with the reduced sample (No. 2) than with the untreated (No. 1). Quantitative data are given in Table 4 for an experiment in which an aliquot of the TCA precipitate was first counted for total counts, the oxidized hydrolysate was then electrophorized on paper as described in Figure 5, and the spots for cysteic acid and alanine were eluted from the paper and counted.

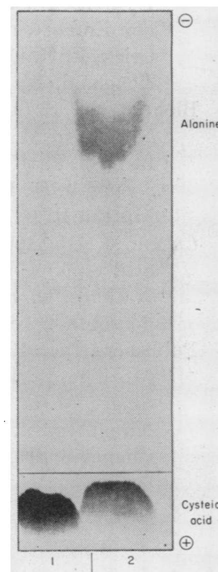


FIG. 5.—Analysis of the radioactivity transferred into TCA-precipitable material. Each sample is the combined Dowex filtrate and eluate from Table 3. The samples were electrophorized on Whatman 3 MM paper at pH 1.85 (acetic acid:formic acid:water, 7.8:2.5:89.7) at 70 volts/cm for 60 min. The electropherogram was stapled to X-ray film and exposed for 25 days. (1) untreated C^{14}-CySH-sRNA^{CySH} (2) Raney Nickel-treated C^{14}CySH-sRNA^{CySH}.

Thus, alanine is accepted by the poly UG system from Ala-sRNA^{CySH}, but not from Ala-sRNA^{Ala}. We conclude, then, that poly UG recognizes the specific sRNA molecule rather than the amino acid.

Discussion.—The results give direct support to the hypothesis that sRNA functions as an adaptor in specifying the fit of an amino acid on a template. Once attached to sRNA, the cysteine could be altered to another one of the natural amino acids, but the coding properties of the hybrid remained unchanged. In other experiments,[16] it has been shown that attached cysteine may also be oxidized to cysteic acid, a rather drastic change in the chemical properties of the amino acid, without loss of reactivity with poly UG. It remains to be shown that the result obtained with the artificial UG polymer carries over to the synthesis of a natural protein. This is currently being tested in the hemoglobin synthesizing system, utilizing reticulocyte ribosomes.

Summary.—A direct test is made of the hypothesis that sRNA functions as an adaptor in specifying the fit of amino acids on a template. Cysteine, attached to its normal acceptor sRNA, is converted, while still attached, to alanine, by reduction with Raney Nickel. The coding properties of the resultant hybrid are examined by the use of poly UG which stimulates incorporation into polypeptide of cysteine but not alanine. Alanine, attached to the cysteine acceptor, is incorporated, indicating that the adaptor hypothesis is correct.

Since an amino acid, once attached, no longer participates in coding, it follows that the code (i.e., the correspondence between nucleotide sequence in template RNA and amino acid sequence in protein) is embodied in the precise structures and interrelationships of the set of sRNA adaptors and activating enzymes.

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† The following abbreviations are used: sRNA, soluble ribonucleic acid; poly-U, polyuridylic acid; poly UG, polyuridylic-guanylic acid; PEP, phosphoenolpyruvate; TCA, trichloroacetic acid.

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