present study has pushed the detection level to 0.01% of the genome of *E. coli*, and it could be decreased further. It is also evident that the use of nucleolytic enzymes will enable the ultimate isolation of specific DNA sequences corresponding to the RNA molecules used for hybridization.

Summary.—Experiments are described that establish the existence of a sequence in *E. coli* DNA complementary to its ribosomal RNA. The proof depends on showing that a hybrid complex, resistant to RNAase, is specifically formed with homologous DNA. The base composition of bacterial ribosomal RNA implies that only one of the two DNA strands is used in the region transcribed.

The procedures developed in the present study provide a method of sufficient specificity and sensitivity to permit the identification, and ultimate isolation, of sequences in DNA complementary to known RNA molecules corresponding to 0.01% of the total genome of *E. coli*.

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† Predoctoral fellow trainee in Molecular Genetics (USPH 2G-319).

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*THE SECONDARY STRUCTURE OF COMPLEMENTARY RNA**

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The existence of enzymes which catalyze the copolymerization of ribonucleoside triphosphates in the presence of a DNA[1] primer is well established.[2–6] In particular, it has been shown that the RNA polymerase of *M. lysodeikticus* catalyzes the formation of an RNA product which has the over-all composition (U substituting

for T) and nearest-neighbor sequence of its DNA template and which contains polynucleotide sequences complementary to its DNA.[6-8] From the extent of complexing of bacteriophage T2 DNA to its complementary RNA, we had tentatively concluded that both DNA strands must serve as a template for C-RNA synthesis.[8] In that case, C-RNA could be self-complementary and, under certain circumstances, might exhibit secondary structure properties like those of native, helical DNA. The experiments described below show this to be the case. During the past decade, the physical chemistry of the natural and synthetic polynucleotides has been the subject of intensive experimentation. As a result of this work, a wide variety of criteria is available for assessing the secondary structure properties of C-RNA. The most restricting of these criteria is that of irreversible denaturation. Only highly ordered polynucleotides of complex sequence can be caused to undergo such irreversible changes of secondary structure.[9] In fact, prior to the work reported here, only DNA had been shown to fall into this exclusive category.[10] Irreversible changes in secondary structure result in readily detectable changes of optical properties, reactivity of functional groups and susceptibility to enzymatic hydrolysis. The demonstration of a DNA-like conformation in C-RNA has accordingly been constructed around a variety of assays of irreversible denaturation.

Experimental Methods.—C-RNA was enzymatically synthesized on a bacteriophage T2 DNA template by a method described previously.[7, 8] Primer DNA and synthesized RNA were separated from protein by phenol extraction.[11] The combined nucleic acids were precipitated by cetyl trimethylammonium bromide or ethanol, redissolved, and separated from each other by 48–72 hr gradient centrifugation in 8.4 molal CsCl at 20–25°C. At the end of that time, the RNA was collected in the densest part of the gradient, precipitated with alcohol, redissolved in 0.01 *M* NaCl at room temperature, and dialyzed against 0.01 *M* NaCl. Samples were stored at −20°C in this solvent. Details have been presented previously.[8] P^{32}-C-RNA was made with ATP or GTP, labeled in the ribose-proximal phosphate. Bovine pancreatic ribonuclease and formaldehyde (reagent grade, 37%) were used without further purification. UV absorbance was measured as a function of temperature in thermostated Beckman DU or Unicam spectrophotometers, using 4.3 × 10 × 38 mm stoppered quartz cells. Changes of concentration resulting from evaporation at the highest temperatures were followed by frequent reweighing, and absorbances were appropriately corrected. The reaction of T2-C-RNA with formaldehyde was also followed spectrophotometrically. RNA and borate buffer were mixed in the stoppered cuvette to give 0.01 *M* borate of pH 8.4 to 8.5, 0.01 *M* NaCl, and the absorbance was measured at 37°C against the appropriate reference buffer. Aliquots of 10% CH_2O in 0.1 *M* borate pH 8.4–8.5 were then added to the RNA solution and reference buffer and mixed, and the cuvettes were returned to the 37°C spectrophotometer as rapidly as possible. After 6–8 hr at 37°C, the RNA-CH_2O solutions were heated at 80°C for 10 min in a stoppered vial, cooled rapidly, and remeasured at 37°C. The sedimentation constants of four T2-C-RNA samples were determined under conditions that would ensure complete dissociation of secondary structure—i.e., in 1% CH_2O, 0.08 *M* NaCl, 0.01 *M* borate buffer, pH 8.3 after 10 min heating to 80°C in this solvent (Table 1). Solutions containing 0.005% RNA were centrifuged at 44,700 rpm, 25°C in a Spinco Model E analytical ultracentrifuge. The conventional UV absorption camera was used with an auxiliary Corning No. 9864 filter. Densitometric traces were made on a Joyce-Loebl recording microdensitometer. The sedimentation constants ($S_{20,w}$) recorded in Table 1 correspond to the rates of migration of the midpoints of absorbance boundaries. Values have been corrected to the viscosity of water at 20°C.

Results and Discussion.—The first indication that T2-C-RNA purified by CsCl density gradient centrifugation might, in part, have a highly ordered secondary structure came from measurements of the absorbance-temperature relation. Figure 1 shows such an experiment. The first heating curve (1) of this T2-C-RNA (sam-

TABLE 1

PROPERTIES OF T2-C-RNA SECONDARY STRUCTURE

Prep.	$S_{20,w}$	Fraction of Nucleotide in Highly Ordered Form (f_h) Heat denaturation	CH_2O	DNA hybrid	RNase	$T_{1/2,d}$*, †	$T_{1/2,i}$*, ‡
9–1	4.3	0.60	—	—	—	63	—
10–15	4.6	0.45	0.42	—	—	63	—
10–24	4.1	0.56	0.50	—	—	61	—
11	4.3	0.66	0.58	—	—	65	—
P^{32}	—	—	—	0.74	0.69	—	61
T2 DNA	(56)§	1.0	—	—	—	59.5	—

* Measured in 0.005 *M* NaCl, 10^{-3} *M* Phosphate, pH 7.1, 10^{-4} *M* EDTA.
† Midpoint denaturation temperature of the ordered form of T2-C-RNA. This is calculated from thermal transition data by the procedure given in footnote 12, and shown in Figure 1, curve 4.
‡ Temperature at which the irreversible denaturation of C-RNA is one half completed (Fig. 4).
§ Measured in 0.9 *M* NaCl, 0.01 *M* Tris pH 7.1.

ple 10–24) in 5×10^{-3} *M* NaCl, 10^{-3} *M* phosphate pH 7.1, 10^{-4} *M* EDTA has two distinct portions: between 15 and 40°C, there is a gradual increase in absorbance (at 260 mμ; A_{260}); near 55°C, the temperature dependence becomes very much greater, and this steep portion of the heating curve ends at about 70°C. If one takes the cuvette which has been heated to 83°C, cools it in ice and reheats, a very shallow absorbance-temperature curve ($A^{(2)}(T)$; Fig. 1, curve 2) is obtained. The absorbance at 25°C has been irreversibly increased by heating to 83°C. This result leads quite naturally to the following interpretation of the initial heating curve (Fig. 1, curve 1): The T2-C-RNA sample contains ordered (helical, DNA-like) and disordered (denatured, TMV-RNA-like) polynucleotide. The gradual increase of A between 15 and 35°C is due to the dissociation of disordered C-RNA secondary structure. At these temperatures, ordered C-RNA secondary structure is stable; its dissociation occurs mainly at 50–70°C. A simple manipulation permits the contribution of these two conformations to the absorbance-temperature curve to be estimated. When this is done, the contribution of disordered C-RNA (Fig. 1, curve 3) can be subtracted from the total absorbance (curve 1) to yield the absorbance-temperature dependence of ordered C-RNA (curve 4). For four samples, the fraction of nucleotides contained in ordered, helical, DNA-like arrays (f_h), ranges from 0.45 to 0.66 (Table 1).[12]

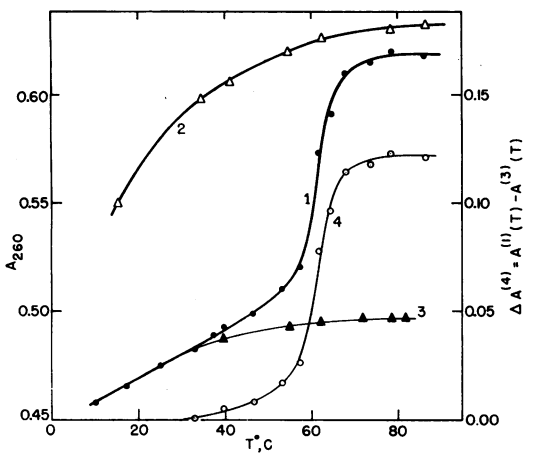

FIG. 1.—Temperature dependence of absorbance at 260 mμ (A_{260}) of C-RNA (sample 10–24) in 5×10^{-3} *M* NaCl, 10^{-3} *M* phosphate, 10^{-4} *M* EDTA, pH 7. Curve 1: Initial heating curve ($A^{(1)}(T)$). Curve 2: The solution from 1 has been cooled rapidly from 83°C to 15°C and is reheated ($A^{(2)}(T)$). Curve 3: $A^{(3)}(T) = A^{(1)}(15) + g[A^{(2)}(T) - A^{(2)}(15)]$. Curves 3 and 1 coincide over the temperature range 15–35°C for $g = 0.44$. Curve 4: $\Delta A^{(4)} = A^{(1)}(T) - A^{(3)}(T)$ (right-hand scale).

The reaction of T2-C-RNA with CH_2O proceeds in a manner that leads qualitatively and quantitatively to the same interpretation. Some of the T2-C-RNA amino groups react with 1 per cent CH_2O at 37°C, pH 8.5. The rest behave like

amino groups in native DNA in that they are made available to CH_2O reaction by heating at 80°C for 10 min (Fig. 2). UV absorption spectra show a shift of the wavelength of maximum absorbance from 258.5 to 260 mμ at 37°C and a further shift to 262 mμ after heating to 80°C. Once again, the relative magnitudes of the

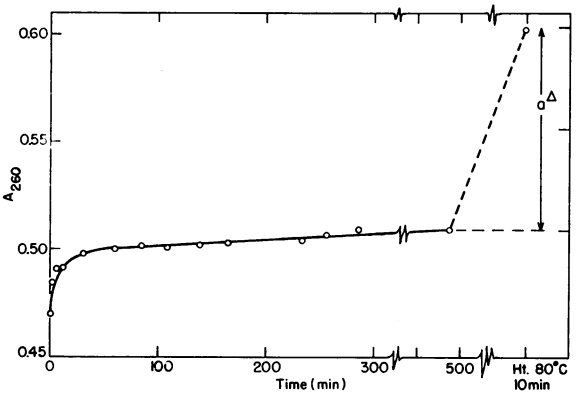

FIG. 2.—Absorbance of C-RNA (sample 10–24) in 1 per cent formaldehyde at 37°C, pH 8.5 as a function of time. After 480 min at 37°C the sample was heated to 80°C for 10 min and remeasured at 37°C. a^{Δ}: absorbance increase on heating to 80°C.

absorbance changes can be used to calculate f_h, the fraction of nucleotide contained in DNA-like arrays. Details of the calculation are given in footnote 13. For the samples shown in Table 1, f_h ranges from 0.42 to 0.58. The formaldehyde reaction values of f_h are consistently lower than f_h calculated from thermal transition data (e.g., Fig. 1). This may be due to a small part of the ordered C-RNA reacting with 1 per cent CH_2O during the 6–8 hr at 37°C that precede the heating.

T2-C-RNA which has been purified in a CsCl density gradient shows a remarkable resistance to digestion by pancreatic RNase (Fig. 3, curve 1). That this property is associated with RNA configuration rather than RNA composition is shown by the dramatic correlation between irreversible denaturation and susceptibility to hydrolysis (Fig. 3). Clearly, the production of acid-soluble fragments by pancreatic RNase constitutes a simple and extremely sensitive assay of C-RNA secondary structure. We have employed it in this manner in following the irreversible thermal denaturation of P^{32}-labeled T2-C-RNA. Figure 4 shows an irreversibility assay of C-RNA secondary structure, the RNase digestion being performed at 37°C after brief heating to a specified temperature. Accordingly, no changes in the release of acid-soluble P^{32} are observed between 25 and 40°C (contrast Fig. 1, curve 1), since ordered C-RNA is not affected in this temperature range and since

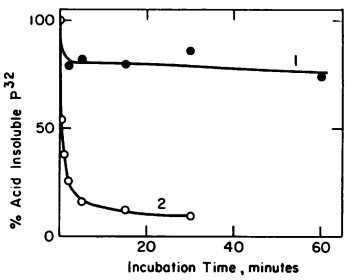

FIG. 3.—Release of TCA-soluble nucleotides from P^{32}-C-RNA by pancreatic RNase at 25°C. Curve 1: T2-C-RNA purified by CsCl gradient centrifugation. Curve 2: Same, after heating RNA at 100°C for 10 min in 0.01 *M* NaCl.

Conditions of RNase assay: The complete system contained 3μmoles of $MgCl_2$; 50 μmoles of Tris, pH 7.81; 1.8 μg of P^{32}-C-RNA (3.33×10^3 cpm per μg) and 0.05 μg of ribonuclease in a total volume of 0.60 ml. This mixture was incubated at 37°. At appropriate time intervals, 0.10 ml aliquots were removed and added to 100 μg of DNA which served as a carrier. The reaction was stopped by the addition of 50 per cent TCA to give a final concentration of 10 per cent, and the mixture was placed in ice. The acid-insoluble material was collected by filtration through membrane filters ("Millipore," Type HA, 0.45 μ pore diameter) and washed three times with successive 5 ml portions of cold 5 per cent TCA. The filters were then dried in planchets and the radioactivity content determined in a gas flow counter.

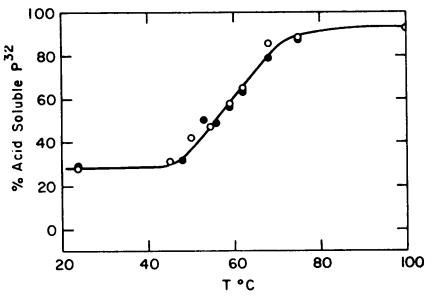

FIG. 4.—Irreversible denaturation of C-RNA in 5×10^{-3} *M* NaCl, 10^{-3} *M* phosphate, 10^{-4} *M* EDTA as detected by susceptibility to pancreatic RNase digestion. Two separate experiments (● and ○) are shown.

Heating procedure: The C-RNA solution (15 μg/ml) is maintained at a given temperature for 10 min. A 0.10 ml aliquot is removed and the remaining solution is equilibrated at the next highest temperature. The percentage of P^{32} rendered acid-soluble by RNase digestion is shown as a function of the heating temperature.

RNase assay: The 0.10 ml aliquot is cooled and made up to a total volume of 1.0 ml with 0.12 ml *M* Tris pH 7.8, 0.06 ml 0.1 *M* $MgCl_2$ and water. Two aliquots are then taken. One 0.45 ml portion is digested with 0.6 μg. RNase for 10 min at 37°C, and acid-precipitable P^{32} is determined. The other aliquot serves as a control of acid precipitable P^{32} before enzymatic digestion.

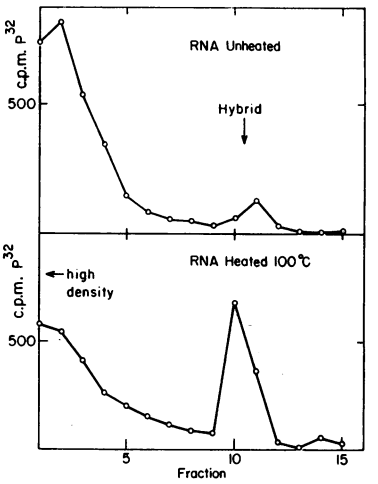

FIG. 5.—Dependence of complex formation between P^{32}-T2-C-RNA and denatured T2-DNA upon RNA secondary structure.

Method: T2-DNA is denatured by heating at 100°C for 10 min in 0.01 *M* NaCl, and quenched. Approximately 100 μg DNA and 15 μg P^{32}-T2-C-RNA are mixed in a total volume of 0.6 ml adjusted to 0.6 *M* CsCl and divided into two parts. One portion of this RNA-DNA mixture is then reheated to 100°C for 15 min and quenched. Both samples are "annealed" at 41.3°C for 8 hr. They are then centrifuged for 72 hr in 3 ml of 8.4 molal CsCl at 25°C, 35,600 rpm. Fractions are collected and TCA insoluble P^{32} is measured as previously described.[8] C-RNA complexed to T2-DNA ("hybrid") appears as a discrete peak of P^{32} radioactivity in the less dense portion of the gradient.

only the *irreversible* structure changes of *ordered* C-RNA are detected in the RNase assay. It should be noted that for such small polynucleotide molecules as these ($S_{20,w}$, Table 1), equilibrium and irreversibility transition assays do not differ appreciably;[15] the corresponding midpoint transition temperatures ($T_{1/2}$, Table 1; Fig. 4; Fig. 1, curve 4) are, indeed, very similar.

If T2-C-RNA preparations isolated by centrifugation in a CsCl density gradient are partly ordered, then the ability to form DNA-C-RNA hybrids with denatured T2 DNA should depend on the thermal history of the C-RNA. This is found to be the case. In the experiment shown in Fig. 5, denatured C-RNA forms four times as much hybrid upon annealing as C-RNA which has not been heated. Moreover, this experiment and the RNase digestion experiment on the same sample are quantitatively consistent: the P^{32}-C-RNA preparation is 3.8 times more effective in complexing T2-DNA (under the conditions of the annealing experiment) after denaturation.[16] It is digested 3.3 times more readily by RNase after denaturation

(Fig. 4); accordingly, one estimates $f_h = 0.74$ from the DNA hybrid experiment and $f_h = 0.69$ from the RNase assay.

Several lines of evidence, as we have seen, suggest that part of our T2-complementary RNA has a secondary structure with stability and other properties very similar to those of ordered, helical DNA. If C-RNA secondary structure were also determined by the interaction of two complementary polynucleotide chains, then heat-denatured T2-C-RNA should be capable of being "annealed" at moderately high temperures, and the rate should depend on concentration.[18, 19] Both of these expectations are borne out. At 55°C in 0.1 *M* NaCl, there is a rapid conversion of heat-denured T2-C-RNA to the RNase-resistant, ordered form. In fact, this "annealed" C-RNA has a higher fraction of ordered nucleotide than the original unheated sample. At RNA concentrations of 200 and 50 μg/ml, the half times of the reaction are 15 and 60 min respectively (Fig. 6). The second-order dependence

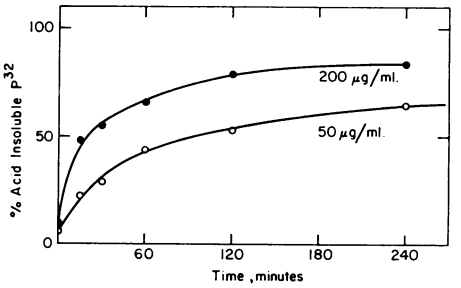

FIG. 6.—Reestablishment of C-RNA secondary structure after thermal denaturation. Ordinate: fraction of RNA not converted to acid-soluble form by incubation with RNase. C-RNA concentration during annealing: ● 200 μg/ml; ○ 50 μg/ml.

Assay: P^{32}-C-RNA is denatured by heating to 100°C for 10 min in 0.01 *M* Tris, pH 7, cooled rapidly in ice and NaCl is added 0.1 *M*. The sample is then placed in a 55.0°C bath. Samples are removed periodically and assayed (method as Fig. 4).

on concentration may be fortuitous, since the time dependence of the annealing process deviates from second order. Such deviations could well arise from heterogeneity among the relatively small C-RNA molecules or from the presence of slightly non-stoichiometric proportions of complementary polynucleotide.

It is important to discover whether C-RNA is synthesized in the highly ordered, DNA-like form. The RNase assay is well suited to deciding this point. We find that C-RNA is very susceptible to RNase hydrolysis at the beginning of its purification and that susceptibility is not greatly increased at this stage by heating at 100°C. For example, when the mixture of RNA and DNA remaining after phenol extraction of the polymerase reaction mixture is digested with RNase (0.6 μg/ml) for 15 min at 37°C, 82 per cent of the C-RNA-P^{32} is rendered acid-soluble. Heating to 100°C and rapid cooling renders 92 per cent of the P^{32} acid-soluble after the same enzymatic digestion. Evidently T2-C-RNA acquires its ordered secondary structure during its lengthy isolation, presumably by intermolecular interactions of the "annealing" type. A different experiment provides independent corroborative evidence on this point. The C-RNA that has been made on a heterogeneous animal DNA primer should also be relatively heterogeneous. If the above interpretation is generally valid and C-RNA is synthesized in a disordered configuration, then any heterogeneous C-RNA that has been isolated by the CsCl procedure should be less highly ordered than similarly purified homogeneous C-RNA, since secondary structure formation by intermolecular "annealing" is less successful in heterogeneous nucleic acids.[19] A preparation of salmon-C-RNA was in fact found to have an f_h of approximately 0.15 (CH_2O assay, data not shown).

Comments.—The experiments described here provide several independent types of

evidence that C-RNA may assume a highly ordered secondary structure in aqueous solution. The properties of C-RNA in this conformation are very similar to those of DNA in that secondary structure transitions occur (Fig. 1) which can only be reversed under special circumstances and involve intermolecular interactions (Figs. 4, 6). This is the first ribopolynucleotide of complex sequence that has been found to possess these properties. In view of the complementarity of T2-C-RNA with its template DNA, it seems overwhelmingly probable that this highly ordered conformation is, in fact, a DNA-like double helix. X-ray diffraction analysis will be needed to make this point absolutely certain. Evidently, the ordered conformation of T2-C-RNA is an accident of the preparative method; the RNA is synthesized in a disordered conformation, and ordering occurs imperfectly during the isolation. Deliberately designed annealing procedures yield a greater degree of ordering.

The self-complementarity of C-RNA has one other significance. Our first experiments on the formation of DNA-C-RNA complexes had led us to believe that both strands of bacteriophage T2 DNA served as templates for C-RNA synthesis. The results reported here provide convincing supportive evidence, and indeed, Chamberlin and Berg have corroborated this point.[5, 20] In fact, the present experiments permit one to be more specific. Not only do both DNA strands serve as template for the RNA polymerase *in vitro*, but they must do so at complementary polynucleotide sequences in order to yield C-RNA chains that are themselves complementary with each other. It is most probable that these complementary DNA polynucleotide sequences are situated at corresponding locations on the two chains.

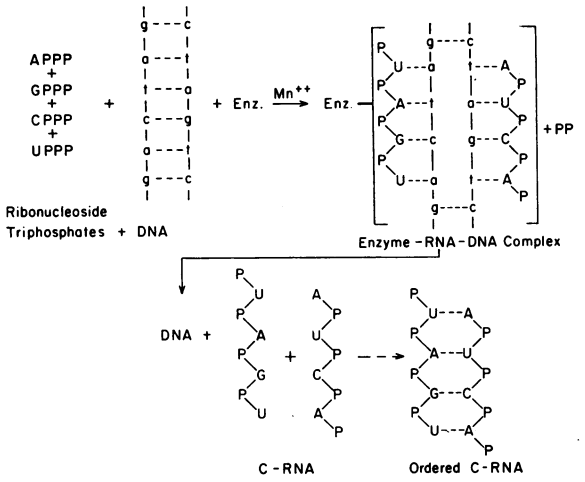

FIG. 7.—Synthesis of complementary RNA. RNA nucleosides are denoted by capital letters, DNA nucleotides by lower-case letters.

It is pertinent to summarize, at this point, the chemical and structural information on primer-product relationships in the reaction catalyzed by RNA polymerase. This is done in the reaction scheme of Figure 7, which incorporates the previously known properties of the RNA polymerase-catalyzed reaction and the results presented in this paper: (1) the requirement for four nucleoside triphosphates, a DNA primer, and divalent metal ions, (2) the correspondence of primer and product nucleotide composition and nearest-neighbor sequence, (3) the relative ease with which DNA primer and C-RNA may be separated from each other, (4) the recovery of native DNA from the reaction mixture, and (5) the self-complementarity of synthesized C-RNA and its ability to assume a highly ordered conformation. The representation of the enzyme-template-product complex, on the other hand, is purely speculative. For example, it is not established (and not probable) that RNA chains may be assembled simultaneously from both strands of a single DNA

molecule; they are shown in this way merely to represent the self-complementarity of C-RNA.

Summary.—(1). The RNA synthesized on a bacteriophage T2 DNA template *in vitro* using the RNA polymerase of *M. lysodeikticus* is self-complementary and can assume a highly ordered, DNA-like secondary structure. In this regard, it is unique among ribopolynucleotides of complex sequence and *qualitatively* different from ribosomal and plant viral RNA's. (2). The ordered C-RNA secondary structure has a stability comparable with that of its homologous DNA. It can be disrupted irreversibly, and also, under suitable circumstances, reformed by "annealing." (3). Pancreatic ribonuclease does not depolymerize ordered C-RNA under conditions that lead to rapid degradation of denatured C-RNA.

We should like to thank T. Nakamoto for most helpful discussions and for preparations of RNA polymerase and RNA.

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† Operated by the University of Chicago for the U.S. Atomic Energy Commission.

[1] The following abbreviations are used: A, adenine; U, uracil; G, guanine; T, thymine; C, cytosine; DNA, sodium deoxyribonucleate; RNA, sodium ribonucleate; C-RNA, "complementary" RNA; TMV, tobacco mosaic virus; ATP, adenosine -5′ triphosphate; GTP, guanosine -5′ triphosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, disodium ethylenediaminetetraacetate; TCA, trichloracetic acid; RNase, ribonuclease.

[2] Weiss, S. B., these PROCEEDINGS, **46**, 1020 (1960).

[3] Stevens, A., *J. Biol. Chem.*, **236**, PC 43 (1961).

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[7] Weiss, S. B., and T. Nakamoto, these PROCEEDINGS, **47**, 1400 (1961).

[8] Geiduschek, E. P., T. Nakamoto, and S. B. Weiss, these PROCEEDINGS **47**, 1405 (1961).

[9] Steiner, R. F., and R. F. Beers, *Polynucleotides* (Elsevier, 1961).

[10] Contrary examples are highly ordered complementary ribopolynucleotide complexes such as poly A + poly U, poly I + poly C, and polydeoxyribo AT and GC, which undergo highly cooperative helix-coil transitions that are completely reversible; they lack the complex nucleotide sequences which make the re-establishment of correctly aligned complementary sequences an improbable event. The complex polynucleotides such as TMV RNA, bacteriophage X174 DNA and poly (AGUC) on the other hand, lack the complementarity that would allow a highly ordered secondary structure to exist. These latter polynucleotides do give ample evidence of intramolecular purine-pyrimidine interactions and undergo relatively broad secondary structure transitions under a variety of circumstances.[9] Evidently, the interactions involve sufficiently short nucleotide sequences that irreversibility does not occur. If these disordered polynucleotides at any time possess a unique biologically significant conformation, it is not one that has thus far been distinguished by enzymological or physical-chemical criteria.

[11] Gierer, A., and G. Schramm, *Z. Naturforschung*, **11 B**, 138 (1956).

[12] The temperature dependence of A for the disordered polynucleotide should be the same as curve 2, Figure 1. Let $g\,A^{(2)}(15)$ represent the absorbance of the original sample (at 15°C) which is contributed by disordered polynucleotide. Then according to the interpretation presented in the text, g is determined by the requirement that for $T < 35$°C, $g[A^{(2)}(T) - A^{(2)}(15)] = A^{(1)}(T) - A^{(1)}(15) = A^{(3)}(T) - A^{(3)}(15)$, i.e., that curves 1 and 3 coincide over the range 15–35°C. For sample 10–24, $g = 0.44$ satisfies that requirement. Curve (4), the difference between curves (1) and (3), is the calculated absorbance-temperature curve of ordered C-RNA. The absorbance contributed by ordered C-RNA at 25°C is then $A_h = A^{(1)}(25) - A^{(2)}(25)$, and the mole fraction of C-RNA in the ordered conformation at 25°C is $f_h = 1 - g$ (estimated reliability ±0.05).

[13] The absorbance change upon heating the CH_2O solution of C-RNA to 80°C (a^{Δ}, Fig. 2) is asseumd to be due to the combined effects of denaturation and formylation. Each of these ef-

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.

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*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.