Enzymatic Synthesis of Ribonucleic Acid

IV. THE DEOXYRIBONUCLEIC ACID-DIRECTED SYNTHESIS OF COMPLEMENTARY CYTOPLASMIC RIBONUCLEIC ACID COMPONENTS*

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The ribonucleic acid product formed by ribonucleic acid polymerase resembles its deoxyribonucleic acid template in base composition and in base sequence (1-6). The reaction in vitro results in the synthesis of RNA chains which are complementary to both DNA strands (6). Evidence for this originates from the formation of highly ordered RNA structures when the RNA polymerase product is annealed with itself (7), and from base composition analysis of the polymerase product when primed by single or double stranded φX174 DNA (8, 9). Self-annealed synthetic complementary ribonucleic acid exhibits properties very similar to helical DNA (7). Although RNA polymerase catalyzes the synthesis of two complementary ribopolymers in vitro, studies with bacteriophage systems have led to the conclusion that informational RNA results from the transcription of only one DNA strand in vivo (10-12). It is not yet certain whether the bacteriophage systems represent a general transcription mechanism or a specialized case. Recently, Hayashi, Hayashi, and Spiegelman (13) reported that when a "replicating form" of φX174 DNA is used in the RNA polymerase reaction in vitro, only one DNA strand is copied.

Observations by other investigators have led to the belief that the synthesis of most, if not all, cytoplasmic RNA is under the direction of DNA. Enucleated cells show a rapid drop in the content of their cellular RNA (14). Actinonycin D, an inhibitor of RNA polymerase, prevents the synthesis of cytoplasmic RNA when given to whole cells (15). Other evidence in support of this concept is the demonstration of specific RNA-DNA hybrid formation between microbial cytoplasmic ribonucleic acids and homologous DNA (16-18). Hybrid formation of this type implies the presence of nucleotide sequences in cytoplasmic RNA which are complementary to base sequences in DNA. Therefore, DNA directs not only the synthesis of template RNA for protein synthesis, but ribosomal and transfer ribonucleic acids as well.

The high degree of self-complementarity among the RNA chains formed by RNA polymerase suggested the possibility that the self-complementary ribonucleic acid product might contain one strand which was identical in base sequence with ribosomal and transfer RNA, and the other strand complementary to these cytoplasmic ribopolymers. A demonstration of synthetic ribonucleic acid complementarity to natural cytoplasmic ribonucleic acids would be evidence that the assembly of polyribonucleotides in vitro results in the synthesis of specific nucleic acid chains, and would strengthen conclusions concerning the origin of cytoplasmic RNA. In this paper, we shall describe experiments which suggest that part of the RNA synthesized on a DNA template, in vitro, is complementary to homologous 23 S and 16 S ribosomal RNA, and to homologous transfer RNA.

EXPERIMENTAL PROCEDURE

Materials and Methods—The RNA polymerase used in this report was purified from extracts of Micrococcus lysodeikticus by Michaelis described previously (19) with the following modifications. Prior to treatment with DEAE-cellulose, ammonium sulfate Fraction V was dissolved in 4 ml of 0.1 M Tris-HCl buffer, pH 7.5, and passed over a Sephadex C-75 column (3.5 × 80 cm) which had previously been equilibrated with 0.3 M ammonium sulfate neutralized with NH₄OH to pH 7.0. The enzyme was eluted at room temperature with this salt solution, and appeared after 150 ml had passed through the column. Ninety per cent of the activity was recovered in a volume of 70 ml, iced, and concentrated by the addition of solid ammonium sulfate to a final concentration of 40% of saturation at 0°. The precipitate was collected by centrifugation and the Sephadex procedure repeated on a second column of equal size. The enzyme collected after the second Sephadex elution was stored in 50% glycerol and treated with DEAE-cellulose as previously reported (19). This procedure significantly reduced the ribonuclease activity associated with the enzyme preparation. On extensive incubation of the enzyme with ³²P-RNA, no loss in acid-insoluble counts was observed. However, some ribonuclease activity still remained as evidenced by a 50% loss in plaque-forming units when 100 μg of enzyme were incubated for 20 minutes at 30° with 0.5 μg of infectious MS₆₂ RNA.¹ This loss in biological activity is equivalent to the action of 5 × 10⁻⁴ μg of pancreatic RNase as determined by assays under identical conditions.

Mammalian, bacterial, and viral DNA and RNA preparations were obtained as described elsewhere (20, 21). Synthetic ¹ MS₆₂ is an RNA-containing coliphage virus which requires the male strain of E. coli, either Hfr or F⁺, as the host for propagation.

² E. coli DNA was supplied by Dr. E. P. Geiduschek, and turnip yellow mosaic virus and tobacco mosaic virus RNA were supplied by Dr. R. Haselkorn.
cRNA was prepared with the purified RNA polymerase and isolated in pure form by equilibrium centrifugation in CsCl as described previously (6).

Growth of Cells and RNA Isolation—Escherichia coli B was grown on the synthetic medium C as described by Roberts, Abelsohn, Cowie, Bolten, and Britten (22). \(^{32} \text{P}\)-Labeled ribosomal and transfer RNA were prepared in a manner similar to that reported by Yanofsky and Spiegelman (16). Cultures of E. coli B in synthetic medium containing 0.02 M sodium phosphate were grown overnight to an optical density at 650 \(\mu\) of approximately 1. The cultures were then diluted in 15 ml of synthetic medium to an optical density of 0.02 and adjusted to contain \(2 \times 10^{-4}\) m inorganic phosphate with 20 \(\mu\) of neutralized, carrier-free \(^{32} \text{P}\). The cells were shaken at 37° for 5 to 6 hours until an optical density of 0.3 to 0.5 was obtained. Inorganic phosphate (1 mmole) was added and the cells were collected by centrifugation, washed once with synthetic medium, and resuspended in 30 ml of synthetic medium containing 0.10 M inorganic phosphate. The cells were grown for one generation, centrifuged, and stored overnight at -20°. This “chase” procedure was used to eliminate \(^{32} \text{P}\)-labeled messenger RNA (23-26).

The frozen pellet was thawed and suspended in 5 ml of a solution containing 0.02 M Tris (pH 7.5), 0.005 M MgCl\(_2\), and 2.5 \(\mu\)g of lysozyme. A solution of 25% Duponol was added to give a final concentration of 1\%, which resulted in a marked increase in viscosity, indicating cell lysis. The nucleic acid was isolated by four or five successive treatments with phenol, followed by three precipitations with ethanol (27). The \(^{32} \text{P}\)-nucleic acid was dried in a vacuum at room temperature for 30 minutes, and then dissolved in a small volume (less than 1 ml) of 0.01 M Tris, pH 7.5.

Transfer RNA—Transfer RNA was isolated from a portion of the \(^{32} \text{P}\)-nucleic acid by chromatography on columns of methylated albumin as outlined by Sueoka and Cheng (28). The labeled RNA fractions used had a single 4 S component on sucrose gradient centrifugation, and were shown to contain 2 to 3% of 16 S RNA fractions used had a single 4 S component on sucrose gradient centrifugation. Each RNA component contains ultraviolet-absorbing material and acid-precipitable radioactivity. The close correspondence of the two profiles throughout the gradient suggests the absence of “unstable” RNA. Each labeled component is completely sensitive to alkali and ribonuclease digestion, and bands in a region distinctly separate from boiling water bath for 10 minutes, and then immediately placed in a 60–65° constant temperature bath and annealed overnight. In later experiments, slow cooling from 85° was used since it resulted in more extensive RNA-RNA complex formation. In some of the early experiments, the mixtures were quenched in ice after heating, just prior to annealing. However, this procedure was discontinued when it was found to have little effect on the amount of hybrid formed.

Assay for RNA-RNA Hybrid Formation—The annealing mixtures were cooled to room temperature and adjusted to 1 ml containing 5 \(\mu\)moles of MgCl\(_2\), 10 \(\mu\)g of pancreatic ribonuclease, and 1 \(\mu\)g of ribonuclease T\(_1\). The vessels were incubated for 1 hour at 37° and then iced. Carrier yeast RNA (1 mg) was added and immediately followed by the addition of 0.10 ml of cold 50% trichloroacetic acid. The acid-insoluble material was then prepared for counting as described elsewhere (19). In the presence of pancreatic ribonuclease alone, the digestion of nonhybridized RNA was incomplete, leading to high base-lines which made detection of small quantities of RNA hybrids difficult. This was especially true in experiments with 4 S \(^{32} \text{P}\)-RNA.

Ribonuclease T\(_1\), prepared from Aspergillus oryzae, was purchased from the Banyko Company, Ltd., Tokyo. Crystalline pancreatic ribonuclease and crystalline lysozyme were purchased from Worthington Biochemicals Corporation.

RESULTS

Fig. 1 shows the distribution of the three major RNA components which appear when E. coli \(^{32} \text{P}\)-RNA is prepared as described under “Experimental Procedure,” and fractionated by sucrose gradient centrifugation. Each RNA component contains ultraviolet-absorbing material and acid-precipitable radioactivity. The close correspondence of the two profiles throughout the gradient suggests the absence of “unstable” RNA. Each labeled component is completely sensitive to alkali and ribonuclease digestion, and bands in a region distinctly separate from

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*The abbreviation used is: cRNA, complementary ribonucleic acid.
Fig. 2. Cesium sulfate equilibrium centrifugation of free and hybridized RNA. Solutions were made up for centrifugation in the SW-39 rotor by mixing 1.05 ml of the nucleic acid solution in 0.05 M Tris-HCl, pH 7.5, with 2.50 ml of saturated cesium sulfate, at 25°, to give a final density of 1.610. Mineral oil (1.9 ml) was layered on the top and the solution was centrifuged at 38,000 r.p.m. for 72 hours at 25° in the model L Spinco ultracentrifuge. Fractions of approximately 0.12 ml were collected from the bottom of each tube. The fractions were analyzed for absorbance at 260 mμ and for acid-precipitable radioactivity before and after RNase treatment. A, unannealed mixture of E. coli DNA (50 μg) and E. coli 23 S 32P-RNA (0.10 μg); B, mixture of T7 bacteriophage crNA (50 μg) and E. coli 23 S 32P-RNA (0.10 μg) annealed overnight at 68°; C, mixture of E. coli crRNA (50 μg) and E. coli 23 S 32P-RNA (0.10 μg) annealed overnight at 68°.

TABLE I
Specific hybrid formation with 23 S and 16 S E. coli 32P-RNA
Annealing mixtures were made up as described under "Experimental Procedure." Each reaction contained 0.10 μg of either 23 S or 16 S 32P-RNA and 16 μg of unlabeled DNA or RNA as indicated. After annealing overnight the mixtures were assayed for RNase-resistant counts.

<table>
<thead>
<tr>
<th>Additions to E. coli 32P-RNA annealing mixtures</th>
<th>23 S 32P-RNA</th>
<th>16 S 32P-RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (no RNase)</td>
<td>43,700</td>
<td>42,060</td>
</tr>
<tr>
<td>None</td>
<td>638</td>
<td>519</td>
</tr>
<tr>
<td>E. coli DNA</td>
<td>3,900</td>
<td>3,154</td>
</tr>
<tr>
<td>E. coli crRNA</td>
<td>6,186</td>
<td>6,316</td>
</tr>
<tr>
<td>T7 DNA</td>
<td>632</td>
<td>778</td>
</tr>
<tr>
<td>T7 crRNA</td>
<td>582</td>
<td>771</td>
</tr>
<tr>
<td>TYMV* RNA</td>
<td>480</td>
<td>548</td>
</tr>
</tbody>
</table>

*TYMV, turnip yellow mosaic virus.

DNA when subjected to cesium sulfate equilibrium centrifugation (Fig. 2A).

When E. coli 23 S 32P-RNA is annealed with T7 crRNA (prepared in vitro with RNA polymerase and T7 DNA) and then subjected to centrifugation in cesium sulfate, the distributions of these heterologous polyribonucleotides are not coincident (Fig. 2B). Treatment of each labeled fraction with ribonuclease shows that the 23 S 32P-RNA is completely degraded. In contrast, when 23 S 32P-RNA is annealed with E. coli crRNA, a different situation is found. After equilibrium centrifugation, both the labeled RNA and the crRNA band in similar positions, and a significant portion of the 23 S 32P-RNA now exhibits resistance to ribonuclease (Fig. 2C).

The experiment in Fig. 2 suggests that certain base sequences in crRNA, prepared under the direction of E. coli DNA in vitro, are complementary to the base sequences of E. coli ribosomal RNA, and that specific hybrids of 23 S RNA with crRNA are formed during annealing. Previous studies in this laboratory have shown that RNA-RNA hybrids are remarkably resistant to enzymatic degradation (7).

Table I shows that both 23 S and 16 S RNA components specifically interact under annealing conditions with homologous DNA and homologous crRNA to form nuclease-resistant products. Annealing with heterologous nucleic acids does not result in the formation of ribonuclease-stable ribopolymers. The more efficient hybridization observed with E. coli crRNA, as compared with E. coli DNA, is probably due to the annealing conditions employed, which favor RNA-RNA rather than RNA-DNA interaction.

Table II further illustrates the specificity of annealing reactions with E. coli ribosomal RNA, and also shows hybrid formation with E. coli 4 S RNA. The complementary ribonucleic acids tested were prepared from the corresponding deoxyribonucleic acids with ribonucleic acid polymerase. It is evident that a significant amount of labeled E. coli 23 S, 16 S, and 4 S RNA, when annealed with E. coli crRNA, is converted to a ribonuclease-resistant form, when compared to annealing mixtures containing heterologous synthetic and natural ribonucleic acids. Only in the case of crRNA of Aerobacter aerogenes was the amount of ribonuclease-resistant material equal to, or slightly greater than, that formed with E. coli crRNA. Table II also shows that after annealing, if the reaction mixture is heated at 100°, quenched in ice, and then treated with ribonuclease, only baseline levels of
acid-precipitable counts remain. Furthermore, E. coli 23 S and 16 S ribonucleic acids are not self-complementary since no resistance to ribonuclease is acquired when these ribopolymers are self-annealed.

**Table II**

Annealing mixtures, made up as described under "Experimental Procedure," contained 0.05 µg of either 23 S, 16 S, or 4 S E. coli αP-RNA and 20 µg of a specified RNA as indicated. The mixtures were annealed overnight and assayed for RNase-resistant counts.

<table>
<thead>
<tr>
<th>RNA additions to annealing mixtures</th>
<th>23 S αP-RNA</th>
<th>16 S αP-RNA</th>
<th>4 S αP-RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli cRNA</td>
<td>2,543</td>
<td>2,265</td>
<td>1,993</td>
</tr>
<tr>
<td>E. coli cRNA (heated and quenched)</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli 23 S RNA</td>
<td>59</td>
<td>82</td>
<td>47</td>
</tr>
<tr>
<td>E. coli 16 S RNA</td>
<td>80</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>A. aerogenes cRNA</td>
<td>2,963</td>
<td>2,727</td>
<td>1,849</td>
</tr>
<tr>
<td>M. lysodeikticus cRNA</td>
<td>65</td>
<td>117</td>
<td>60</td>
</tr>
<tr>
<td>Bacillus subtilis cRNA</td>
<td>63</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Bacteriophage T7 cRNA</td>
<td>53</td>
<td>61</td>
<td>52</td>
</tr>
<tr>
<td>Calf thymus cRNA</td>
<td>72</td>
<td>57</td>
<td>81</td>
</tr>
<tr>
<td>Bacteriophage MS2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>50</td>
<td>122</td>
<td>83</td>
</tr>
<tr>
<td>None (no ribonuclease treatment)</td>
<td>19,784</td>
<td>17,961</td>
<td>22,570</td>
</tr>
</tbody>
</table>

**Fig. 3 (left).** The formation of RNA-RNA hybrids as a function of time. E. coli cRNA (80 µg) and 0.5 µg of either 4 S or 23 S αP-RNA were mixed and adjusted to contain 0.2 M NaCl and 0.01 M Tris-HCl, pH 7.5, in a final volume of 2 ml. The mixture was heated for 5 minutes at 100°, quenched in ice, and then maintained at 65° in a water bath. Aliquots of 0.15 ml were removed at specified time intervals, quenched in ice, and assayed for RNase-resistant counts as described under "Experimental Procedure."

**Fig. 4 (right).** The effect of temperature on RNA-RNA hybrid formation. Annealing mixtures were made up as described under "Experimental Procedure." Each mixture contained 10 µg of E. coli cRNA and 0.5 µg of either E. coli 4 S or 23 S αP-RNA. Annealing was carried out overnight at the specified temperatures. Hybrid formation was determined by the RNase assay technique described under "Experimental Procedure."
as well as heterologous tobacco mosaic virus RNA, have little or no effect on the amount of labeled hybrid formed. Ribonuclease-resistance counts were determined in a similar manner except that annealing and ribonuclease digestion were omitted.

The RNA was then subjected to alkaline hydrolysis, and base composition was determined by examining the $^{32}$P-nucleotide content after nucleotide separation by paper electrophoresis as previously described (5). Base compositions of unhybridized 23 S and 4 S $^{32}$P-RNA were determined in a similar manner except that cytoplasmic ribonucleic acids are synthesized on DNA templates and that separate cistrons code for 16 S and 23 S ribosomal RNA.

For a given quantity of cRNA, there should be a limited number of sites available for association with each cytoplasmic RNA component. The level at which saturation of these sites occurs can be determined by annealing with increasing amounts of RNA to the annealing mixtures were made as indicated. 

<table>
<thead>
<tr>
<th>Base</th>
<th>DNA*</th>
<th>21 S $^{32}$P-RNA used for annealing</th>
<th>21 S $^{32}$P-RNA hybridized with cRNA</th>
<th>4 S $^{32}$P-RNA used for annealing</th>
<th>4 S $^{32}$P-RNA hybridized with cRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>mole %</td>
<td>mole %</td>
<td>mole %</td>
<td>mole %</td>
<td>mole %</td>
</tr>
<tr>
<td></td>
<td>24.9</td>
<td>21.8</td>
<td>22.3</td>
<td>27.7</td>
<td>25.6</td>
</tr>
<tr>
<td>A</td>
<td>24.8</td>
<td>27.3</td>
<td>28.2</td>
<td>19.5</td>
<td>20.8</td>
</tr>
<tr>
<td>G</td>
<td>24.9</td>
<td>30.6</td>
<td>29.6</td>
<td>31.0</td>
<td>31.7</td>
</tr>
<tr>
<td>U (1)</td>
<td>20.4</td>
<td>20.3</td>
<td>19.8</td>
<td>21.8</td>
<td>21.9</td>
</tr>
</tbody>
</table>

* Base proportions as reported by Josse, Kaiser, and Kornberg (30).

The specific dilution of E. coli 16 S $^{32}$P-RNA-cRNA hybrid with E. coli 16 S RNA

<table>
<thead>
<tr>
<th>Additions to E. coli 16 S $^{32}$P-RNA + cRNA annealing mixtures</th>
<th>Dilation factor for 16 S $^{32}$P-RNA</th>
<th>RNase-resistant counts after annealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3744</td>
<td>100</td>
</tr>
<tr>
<td>Omit cRNA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. coli 23 S RNA (0.10 pg)</td>
<td>3</td>
<td>1784</td>
</tr>
<tr>
<td>E. coli 16 S RNA (0.10 pg)</td>
<td>0</td>
<td>3460</td>
</tr>
<tr>
<td>E. coli 4 S RNA (0.10 pg)</td>
<td>0</td>
<td>2505</td>
</tr>
<tr>
<td>Tobacco mosaic virus RNA (0.10 pg)</td>
<td>0</td>
<td>3800</td>
</tr>
</tbody>
</table>

* Percentage values are corrected for base-line counts obtained when cRNA was omitted.

The conditions of annealing were as described in "Experimental Procedure." Each annealing mixture contained 16 pg of E. coli cRNA and 0.05 pg of E. coli 23 S $^{32}$P-RNA. Additions of unlabeled RNA to the annealing mixtures were made as indicated. Ribonuclease-resistance counts were determined as outlined under "Experimental Procedure." Table IV except that the annealing mixture contained 0.05 pg of E. coli 16 S $^{32}$P-RNA.

The experimental conditions were the same as indicated for Table IV except that the annealing mixture contained 0.05 pg of E. coli 16 S $^{32}$P-RNA. Table V except that the annealing mixture contained 0.05 pg of E. coli 4 S $^{32}$P-RNA.

The specific dilution of labeled 23 S, 16 S, and 4 S RNA makes it unlikely that contaminating $^{32}$P-messenger RNA accounts for hybrid formation, since this component would not compete for the same sites on cRNA as the 23 S, 16 S, and 4 S diluents. These data support the notions that cytoplasmic ribonucleic acids are synthesized on DNA templates and that separate cistrons code for 16 S and 23 S ribosomal RNA.
labeled cytoplasmic RNA until a plateau in ribonuclease-resistant counts is reached. Fig. 5A demonstrates the saturation plateau for two levels of E. coli cRNA determined by annealing with increasing amounts of E. coli 23 S $^{32}$P-RNA. The level of saturation is directly proportional to the amount of cRNA used, while the ratio of ribonuclease-resistant counts to cRNA employed remains constant. Saturation is reached when 0.10 to 0.12% of the cRNA sequences are hybridized with 23 S $^{32}$P-RNA. When saturation levels were determined for E. coli DNA, by annealing with 23 S $^{32}$P-RNA in the same manner, a similar value of 0.11% was found (Fig. 5B).

The determination of saturation for E. coli cRNA with 4 S $^{32}$P-RNA is shown in Fig. 6. Saturation of E. coli cRNA with E. coli 4 S $^{32}$P-RNA E. coli cRNA (7.3 μg) was annealed with increasing quantities of 4 S $^{32}$P-RNA and assayed for RNase-resistant counts as described under "Experimental Procedure.""%Added recoverable counts hydrolyzed

Fig. 7. Irreversible thermal denaturation of 4 S and 23 S RNA hybrids. 4 S RNA hybrid: E. coli cRNA (80 μg) and E. coli 4 S $^{32}$P-RNA (0.8 μg) were annealed overnight at 65° in 2 ml of 0.2 M NaCl-0.01 M Tris-HCl, pH 7.5, and then diluted to 45 ml with a solution containing 7 X 10$^{-4}$ M Tris-HCl, pH 7.5, and 8 X 10$^{-4}$ M Versene. This solution was divided into three separate portions and each adjusted to contain 0.006 M, 0.05 M, and 0.20 M NaCl, respectively. Samples (1 ml) were maintained at a given temperature for 15 minutes, quenched in ice, and assayed for RNase-resistant counts as described under "Experimental Procedure." 23 S RNA hybrid: the protocol for this experiment was identical with the one described above except that 0.8 μg of E. coli 23 S $^{32}$P-RNA was used instead of 4 S $^{32}$P-RNA.

Previous work with a cRNA-cRNA hybrid indicated that the extent of ordered conformation could be ascertained by a study of its thermal stability (7). When this complex is thermally dissociated it becomes susceptible to nuclease digestion. The
**DISCUSSION**

The conclusions derived from this study rest on the assumptions that (a) the nucleic assay technique can distinguish between ordered RNA and randomly coiled RNA, and (b) RNA-RNA hybrids can be established only between RNA molecules which have reasonably large regions of sequence complementarity. A previous study of annealed cRNA indicated that it had physical properties similar to native DNA and was resistant to ribonuclease. Its ordered structure appears to protect the polyribonucleotide from ribonuclease cleavage whereas disordered RNA (heat denatured) readily undergoes digestion. The nuclease resistance of RNA may, therefore, be used to distinguish between randomly coiled RNA and RNA hybrids. The degree of resistance is a measure of the extent of ordered regions, which in turn depends upon sequence complementarity. The advantage of this technique over the more precise and elegant optical method for determining secondary structure, as first exploited by Doty et al. (31), is that (a) it is simple and requires no special equipment and (b) by the use of highly labeled RNA extremely small quantities of the RNA-RNA species may be detected even in the presence of relatively large amounts of single stranded RNA molecules.

The experiments reported here show that the annealing of radioactive cytoplasmic polyribonucleotides (23 S, 16 S, and 4 S RNA) with homologous synthetic cRNA results in the formation of ribonuclease-resistant 32P-RNA. Substitution of homologous cRNA by heterologous synthetic or natural ribonucleic acids in similar annealing reactions, produces no appreciable protection of the labeled RNA from enzymatic degradation. Similar findings have been reported for the annealing of 32P-cytoplasmic RNA with homologous DNA (16-18). The effectiveness of the RNase technique in distinguishing *bona fide* complementary RNA sequences from accidental sequence homology over short regions, or simple mechanical trapping, is illustrated by the marked specificity with which hybrid formation occurs between *E. coli* cRNA and homologous 23 S, 16 S, and 4 S 32P-RNA (Tables I and II). The negative cases found are particularly impressive. *Aerobacter* cRNA showed significant association with all three species of *E. coli* cellular 32P-RNA. These microorganisms are classified in the same family of Enterobacteriaceae (32). Our results suggest that sequence homology occurs in ribosomal and transfer ribonucleic acids from organisms that are genetically related. Goodman and Rich have reported on cross-complementarity between *E. coli* 32P-soluble ribonucleic acid and deoxy-ribonucleic acid, obtained from closely related species (17).

The term $T_m^*$ as used here, refers to the temperature of incubation which renders 50% of the RNA hybrid susceptible to ribonuclease hydrolysis.
RNA molecules which are complementary as well as identical in base sequence with cytoplasmic RNA components. The results support the idea that ribosomal and transfer ribonucleic acids are synthesized on a DNA template by the action of RNA polymerase.

The specific blocking of 23 S, 16 S, and 4 S 32P-RNA-cRNA hybrid formation with unlabeled cytoplasmic ribonucleic acids of only the same molecular species indicates that each RNA component associates with different sites in cRNA. Approximately 0.11% of the cRNA sequences are complementary to 23 S RNA and 0.014% are complementary to 4 S RNA.

REFERENCES
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