Bacterial Proteins Required for Replication of Phage Qβ Ribonucleic Acid

PURIFICATION AND PROPERTIES OF HOST FACTOR I, A RIBONUCLEIC ACID-BINDING PROTEIN*

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SUMMARY

A bacterial protein (host factor I) required for the replication of bacteriophage Qβ RNA in vitro has been purified from uninfected Escherichia coli to apparent homogeneity. When analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate the purified protein migrates as a single band with an apparent molecular weight of 12,500. The molecular weight of the active protein is approximately 75,000, suggesting a subunit structure composed of six polypeptide chains. RNA synthesis catalyzed by the Qβ RNA polymerase with Qβ RNA as template is completely dependent upon the presence of the purified protein. The concentration of factor required for the maximum rate of synthesis varies according to the amount of Qβ RNA added, but is independent of enzyme concentration. The stoichiometry of the reaction suggests that 1 molecule of factor is required per molecule of RNA. Binding of factor to several different types of single stranded RNA was demonstrated by zonal centrifugation; binding was not detected with double stranded reovirus RNA or with double or single stranded DNA. Studies on the role of factor in the reaction suggest that this host protein acts as a positive control element required at some step prior to the initiation of synthesis with Qβ RNA template.

EXPERIMENTAL PROCEDURE

Materials

Qβ RNA Polymerase—Qβ RNA polymerase was purified and analyzed as previously described (6, 7). One unit of enzyme is that amount catalyzing the incorporation of 1 nmole of GMP per 20 min at 37° in a reaction mixture containing Qβ RNA as template and an excess of HF1 and HF11 (6). When analyzed by polyacrylamide gel electrophoresis in the presence of SDS these enzyme preparations were found to contain only the four polypeptides which have been reported by others to be associated with purified enzyme (4, 5), presumably as enzyme subunits.

Host Factor I—HF11 was purified by either of two methods. The initial studies described here utilized a partially purified preparation (hydroxylapatite fraction) of HF1 and enzyme activity, prepared from phage Qβ-infected E. coli Q13 as previously described (2). More recently a highly purified preparation was obtained from uninfected E. coli Q13 as described by Kuo (8).

The abbreviation used is: SDS, sodium dodecyl sulfate.
**Nucleic Acids**—The RNA of Q3, f2, and R23 coliphages was prepared by phenol extraction and ethanol precipitation (6). *E. coli* tRNA was prepared by phenol extraction of ribosomes isolated by high speed centrifugation (9). Reovirus RNA was isolated from purified virus (10) provided by Dr. A. R. Bellamy. After phenol extraction in the presence of 1% SDS the RNA was further purified by zonal centrifugation. Fractions sedimenting at approximately 10 S were pooled, concentrated by ethanol precipitation, and dissolved in 50 mM Tris-acetate buffer, pH 7.7, and 2 mM EDTA. This RNA showed a sharp hyperchromic thermal transition at 77° in 15 mM sodium citrate-150 mM sodium chloride suggesting that at least 90% was double stranded.6 fd DNA, prepared according to the method of Marvin and Schaller (11), was a gift of Dr. Paul Sadowski; λ Ctt DNA, provided by Dr. David Axelrod, was prepared by a slight modification of the method of Baldwin et al. (12); PBSX DNA, purified as described by Okamoto et al. (13), was a gift of Dr. W. Huang.

**Chemicals**—All chemicals were purchased from standard sources. [γ-32P]GTP was prepared as previously described (14) with the use of GTP purified as described by Hurlbert and Furlong (15) to remove contaminating nucleotides.

**Methods**

**Protein Concentration**—Samples were analyzed by the method of Lowry et al. (16) with bovine serum albumin used as standard. When a more sensitive method was required the protein was hydrolyzed by incubation in 20% NaOH for 20 min at 110°, and the amino acid concentration was measured with ninhydrin by the method of Hill and Delaney (17). L-Leucine was used as standard. Samples containing ammonium sulfate were first dialyzed extensively against 25 mM NaCl and 4 mM 2-mercaptoethanol.

**Amino Acid Analysis**—The factor preparations (glycerol gradient fractions) were dialyzed for 7 hours against 25 mM NaCl and 4 mM 2-mercaptoethanol in order to remove ammonium sulfate. They were then adjusted to 6 M HCl and 0.5% 2-mercaptoethanol and hydrolyzed in evacuated sealed tubes at 100° for 22 hours. The amino acid analysis was performed in a Spinco 1203 analyzer equipped with 6.6 mm cells and a high sensitivity recorder.

**Carbohydrate Analysis**—Samples (30 to 30 μg of protein) were analyzed by gas-liquid chromatography according to the method of Albersheim et al. (18), with inositol as an internal standard.

**Polyacrylamide Gel Electrophoresis**—Samples of protein were first dialyzed against 10 mM sodium phosphate buffer, pH 7.0, for approximately 2 hours. They were then adjusted to contain 1% SDS, 1% 2-mercaptoethanol, and 10% sucrose, and heated to 100° for 1 min. Electrophoresis was performed as described by Maizel (19) using 10% acrylamide gels at 70 volts constant voltage (7 to 12 mA per gel) for 3 hours. Protein bands were stained at room temperature with 0.05% Coomassie brilliant blue in 12.5% trichloroacetic acid for at least 12 hours and destained with 7% acetic acid. The molecular weight of HF1 was estimated as described by Shapiro et al. (20), by comparing its mobility with that of proteins of known molecular weight.

**Assay of Host Factor I**—The assay measures the incorporation of [3H]AMP into acid-insoluble material in the presence of Q3 RNA, the Q3 RNA polymerase, and HF1. With the purified Q3 RNA polymerase and the partially purified or highly purified HF1, RNA synthesis with Q3 RNA template was completely dependent on added HF1 (2). The assay mixture (0.1 ml) contained 100 mM Tris-HCl buffer, pH 7.6, 10 mM MgCl2, 4 mM 2-mercaptoethanol, 1 mM each of UTP, GTP, and ATP, and [3H]AMP, 1 pmoles of Q3 RNA, HF1 in excess (1 to 2 μg of protein of the purified fraction), 1.0 unit of the Q3 RNA polymerase, and an appropriate amount of HF1. After incubation for 20 min at 37° the reaction was terminated by adding 5% trichloroacetic acid containing 20 mM PPi, and the acid-insoluble material was collected and washed on membrane filters as previously described (6). Reaction mixtures lacking HF1 served as blanks. The activity was routinely measured at several protein concentrations to establish a linear relationship between protein concentration and nucleotide incorporation (0.1 to 0.5 unit of HF1 with 1 unit of polymerase). One unit of HF1 is calculated from this linear range as that amount corresponding to the incorporation of 1 n mole of AMP under these standard conditions.

**Association of Host Factor I with Polynucleotides**—The binding of HF1 to nucleic acids was carried out in a reaction mixture containing 100 mM Tris-HCl buffer, pH 7.6, 10 mM MgCl2, 50 mM KCl, 4 mM 2-mercaptoethanol, 30 to 60 μg of polynucleotide, and approximately 35 units of HF1 in a total volume of 0.1 ml. After mixing at 0 to 4° the sample was layered on a solution containing a linear gradient of 5 to 30% glycerol, 100 mM Tris-HCl buffer, pH 7.8, 10 mM MgCl2, 50 mM KCl, and 4 mM 2-mercaptoethanol and centrifuged in a Spinco SW 65 rotor as indicated in the legends. Fractions (0.25 ml) were collected from the bottom of the tube and analyzed for absorbance at 260 nm and for HF1 activity.

**Pyrophosphate Exchange Reaction**—The reaction mixture (0.25 ml) contained 100 mM Tris-HCl buffer, pH 7.8, 10 mM MgCl2, 4 mM 2-mercaptoethanol, 0.8 mM ATP, UTP, GTP, and CTP, 1.5 mM Na32PPi (specific activity 7.4 × 108 cpm per μmol), 1.3 pmoles of Q3 RNA, 2 units of the Q3 RNA polymerase, 2 μg of HF1 (hydroxylapatite fraction), and HF1 as indicated. After incubation at 37° for 20 min the reaction was terminated by the addition of approximately 3 ml of 10% trichloroacetic acid containing 0.2 μM Na32P. Norit (0.1 ml of a 30% solution) was added with occasional mixing and after 10 min the suspension was filtered through a Millipore filter and washed 4 times with approximately 3 ml of 0.2 M Na32P. The filters were glued to planchets with the Norit facing downward and dried. The radioactivity was measured in a gas flow counter.

**Synthesis of o-Terminal Oligonucleotide of Complementary Strand**—The reaction mixture (0.1 ml) contained 100 mM Tris-HCl buffer, pH 7.8, 10 mM MgCl2, 4 mM 2-mercaptoethanol, 0.3 mM each of ATP and [3H]GTP, 3 units of Q3 RNA polymerase, 2 pmoles of Q3 RNA, 0.3 μg of purified HF11, and 0.6 μg of HF1 where indicated. After incubation for 20 min at 37° the reaction was terminated by cooling on ice and the sample was applied to a column (0.25 × 6 cm) of Sephadex G-15 (fine) and eluted with a buffer solution containing 100 mM Tris-HCl, pH 7.8, 10 mM MgCl2, and 4 mM 2-mercaptoethanol. Fractions were collected in scintillation vials, and radioactivity was measured after addition of 15 ml of Bray's solution (21).

**[γ-32P]GTP Incorporation**—The reaction mixtures (0.2 ml) contained 100 mM Tris-HCl buffer, pH 7.8, 10 mM MgCl2, 4 mM 2-mercaptoethanol, 0.15 mM each of [γ-32P]GTP and ATP, 4 units of Q3 RNA polymerase, 1.1 pmoles of Q3 RNA, 0.2 μg of purified HF11, and either none or 0.4 μg of HF1. A reaction mixture con-
FIG. 1. Purification of HF1 by zone sedimentation. The dialyzed QAE-Sephadex fraction (0.3 ml) was centrifuged in a linear gradient of 5% to 20% glycerol as described in the text. Protein (●) was measured with ninhydrin after alkaline hydrolysis, and HF1 activity (○) as described under "Methods." The arrow indicates the location of bovine serum albumin (mol wt = 67,000).

TABLE I

Purification of host factor I

HF1 was purified as described in the text. Protein was determined by the method of Lowry et al. (16) in Fractions 1 to 4, and with ninhydrin (17) after alkaline hydrolysis in Fractions 5 and 6. Numbers in parentheses are approximate due to inhibitors in these fractions.

| Fraction     | Units | Protein | Specific activity |
|--------------|-------|---------|------------------|
|              | mg    | units/mg protein |
| 1. Crude     | (34,000) | 7,000 | (5) |
| 2. Phase     | (28,000) | 3,600 | (8) |
| 3. Heated    | 21,000 | 265   | 70   |
| 4. DEAE-cellulose | 8,700 | 19 | 490 |
| 5. QAE-Sephadex | 5,500 | 0.8 | 6,900 |
| 6. Glycerol gradient | 4,700 | 0.3 | 16,000 |

The bacteria were grown to a concentration of 1 x 10^9 cells per ml in 50 liters of a medium containing (per liter) 1.21 g of Tris base, 5.0 g of NaCl, 1.0 g of NH₄Cl, 52 mg of Na₂HPO₄, 7H₂O, 100 mg of MgSO₄, 10 g of casaminio acids, 10 ml of glycerol, and 2.5 ml of 1.0 M HCl. The cells were harvested in a Sharples continuous flow centrifuge, frozen without washing, and stored at -20°C. Unless otherwise indicated, subsequent operations were carried out at 0-4°C.

Preparation of Crude Extract—Frozen cells (100 g) and cold alumina (200 g) were ground in a chilled mortar. The cell paste was suspended by gradual addition of 400 ml of a buffer solution containing 50 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 5 mM 2-mercaptoethanol, and 1 M NaCl. The suspension was centrifuged at 30,000 x g for 5 min. The supernatant was retained (crude fraction, 390 ml).

Liquid Polyethylene Fractionation—To 390 ml of the crude extract were added with mixing 42 ml of 20% (w/w) dextran 300, 112 ml of 50% (w/w) polyethylene glycol, and 61 g of NaCl. Mixing was continued for 1 h, and the suspension was then centrifuged at 30,000 x g for 5 min. The upper phase was retained (phase fraction, 415 ml).

Heat Treatment—The phase fraction was heated in a boiling water bath with mixing until the temperature of the suspension reached 82-85°C (5 to 7 min) and then immediately cooled on ice. The suspension was centrifuged at 30,000 x g for 15 min, and the supernatant was retained (heated fraction, 355 ml).

DEAE-cellulose Chromatography—The heated fraction was dialyzed against 12 liters of a standard buffer solution containing 50 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, and 5 mM 2-mercaptoethanol for 3 to 4 h. The heated fraction was then centrifuged at 30,000 x g for 15 min, and the supernatant was retained (heated fraction, 355 ml).

DEAE-cellulose Chromatography—The heated fraction was dialyzed against 12 liters of a standard buffer solution containing 50 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, and 5 mM 2-mercaptoethanol for 3 to 4 h. The heated fraction was then centrifuged at 30,000 x g for 15 min, and the supernatant was retained (heated fraction, 355 ml).

QAE-Sephadex Chromatography—The DEAE-cellulose fraction was dialyzed against 4 liters of the standard buffer solution containing 70 mM (NH₄)₂SO₄ and applied to a column (2 x 20 cm) of QAE-Sephadex A-50 previously equilibrated with the same buffer solution. A linear gradient of 100 ml to 300 ml (NH₄)₂SO₄ in a total volume of 450 ml of the standard buffer solution was then applied, and a flow rate of 15 ml per hour was maintained by slight positive pressure. HF1 activity was measured in a single peak eluting between 170 and 225 ml (NH₄)₂SO₄; the bulk of the contaminating proteins eluted at the beginning of the salt gradient. In order to concentrate the factor the active fractions (130 ml) were pooled, dialyzed against 2 liters of the standard buffer solution containing 70 mM (NH₄)₂SO₄, and added at 10 ml per hour to a column (1 x 2 cm) of QAE-Sephadex A-50. HF1 was eluted from the column with 7 to 10 ml of 1 M (NH₄)₂SO₄ in standard buffer and concentrated by dialysis against Sephadex G-200 (QAE-Sephadex fraction, 1.5 ml).

Zone Sedimentation—The QAE-Sephadex fraction was dialyzed against 0.2 M (NH₄)₂SO₄ in the standard buffer solution for 2 h. Aliquots of 0.2 to 0.4 ml were layered on linear gradients.
FIG. 2. SDS-polyacrylamide gel electrophoresis of HFI. The proteins examined were 30 μg of the QAE-Sephadex fraction (left) and 15 μg of the glycerol gradient fraction (right). Electrophoresis was carried out as described under "Methods." The direction of migration was from top to bottom.

of 5 to 20% glycerol in standard buffer solution containing 0.2 M (NH₄)₂SO₄ and centrifuged in an SW 65 rotor at 50,000 rpm for 17 to 20 hours. Fractions (0.25 ml) were collected from the bottom of the tube. Almost all of the protein sedimented as a single component coincident with factor activity (Fig. 1). The active fractions were pooled (glycerol gradient fraction, 3.5 ml).

Comments on Purification Procedure—A summary of the purification procedure is shown in Table I. Precise determination of factor activity in the crude and phase fractions was not possible because of inhibitors of the reaction present in these fractions. The purified HFI (glycerol gradient fraction) was stable for at least 1 month at 0-4°C. However, factor activity is rapidly lost at low concentration (less than 10 μg per ml of protein). Because HFI is heat stable, it is possible to remove contaminating proteins by heat treatment at an early step in the purification procedure without significant loss of activity. However, to determine whether heat treatment modified the physical properties of the factor, a second purification procedure, avoiding the heat step, has been developed. This procedure, utilizing phosphocellulose chromatography, is reported elsewhere (22). The specific activity of the purified factor, its molecular weight, clution from QAE-Sephadex, and migration during SDS-polyacrylamide gel electrophoresis were the same for both preparations.

Concentration of HFI by (NH₄)₂SO₄ precipitation was avoided since this invariably resulted in a loss of activity.

Properties of Host Factor I

Protein Purity—Factor activity was found coincident with protein in the fractions recovered from zonal centrifugation, the final step in the purification procedure (Fig. 1). Upon analysis of this purified fraction by SDS-polyacrylamide gel electrophoresis only a single protein band was detected (Fig. 2). This protein was the major component of the QAE-Sephadex fraction as well. The purified factor was free of RNase activity as measured by sedi-

TABLE II

Amino acid composition

The results are the average of four determinations using three different factor preparations. Molar ratios were calculated relative to alanine. The number of residues per chain was calculated assuming a molecular weight of 12,500; the values have been rounded to the nearest integer. Tryptophan and cysteine were not determined.

| Amino acid | Molar ratio | Mole percent | Residues per chain |
|------------|-------------|--------------|--------------------|
| Lysine     | 0.6         | 3.8          | 4                  |
| Histidine  | 0.6         | 3.8          | 4                  |
| Arginine   | 0.6         | 3.8          | 4                  |
| Aspartic acid | 1.5   | 6.6          | 10                 |
| Threonine  | 0.9         | 5.8          | 6                  |
| Serine     | 2.2         | 14.0         | 14                 |
| Glutamic acid | 2.1 | 13.5         | 13                 |
| Proline    | 0.6         | 3.8          | 4                  |
| Glycine    | 1.3         | 8.3          | 8                  |
| Alanine    | 1.0         | 6.4          | 6                  |
| Cysteine   |             |              |                    |
| Valine     | 1.3         | 8.3          | 8                  |
| Methionine | 0.1         | 0.6          | 1                  |
| Isoleucine | 0.7         | 4.5          | 4                  |
| Leucine    | 1.1         | 7.0          | 7                  |
| Tyrosine   | 0.5         | 3.2          | 3                  |
| Phenylalanine | 0.5 | 3.2         | 3                  |
| Tryptophan |             |              |                    |
| Total      | 15.6        |              | 96                 |
FIG. 4. Requirement for HF₁ as a function of enzyme or RNA concentration. Reactions in 0.1 ml volume were carried out for 20 min with excess HF₁ (2 µg of hydroxylapatite fraction) and varying HF₁ concentrations as shown. The molar concentration of HF₁ was based on a molecular weight of 75,000 and an estimated purity of 80% for the factor preparation. A, RNA concentration constant (1.1 pmoles per reaction) with varying enzyme concentrations: 0, 0.1 unit; △, 0.2 unit; and •, 0.5 unit. B, enzyme concentration constant (0.5 unit) with varying RNA concentrations: O, 0.22 pmoles; △, 0.44 pmoles; and •, 1.1 pmoles.

FIG. 5. Analysis of the association of HF₁ and Q₈ RNA by zone sedimentation. The samples were centrifuged at 100,000 rpm for 2 hours as described under "Methods." A, Q₈ RNA; B, HF₁; C, Q₈ RNA and HF₁. O—O, absorbance; ▲—▲, HF₁ activity.

that of a protein, as determined by density gradient sedimentation in CsCl.

Studied on Role of Host Factor I

Requirement for Host Factor I—Replication of Q₈ RNA is completely dependent on HF₁ (Fig. 3). The small amount of RNA synthesized after prolonged incubation in the absence of factor is 6 S RNA, which can be replicated in the absence of host factors (23). Synthesis of this RNA is presumably due to contamination of the enzyme preparation with a small amount of 6 S RNA, since it is also observed when the Q₈ RNA template is omitted from the reaction mixture.

At low concentrations of HF₁, the rate of synthesis is proportional to factor concentration. At high factor concentrations, the reaction is terminated after an initial lag, and this rate-limiting step in the reaction is consistent with the requirement for HF₁.

Requirement for Host Factor I as Function of RNA Concentration—The concentration of factor required for the maximum rate of synthesis varies according to the amount of RNA added to the reaction, but is independent of enzyme concentration (Fig. 4). The amount of factor required for half-maximal activity was the same over a 5-fold range of enzyme concentrations when the amount of RNA in the reaction was held constant (Fig. 4A). With different concentrations of RNA and constant enzyme, the amount of factor required for half-maximal activity was proportional to the amount of RNA (Fig. 4B). These data suggest that the role of HF₁ in the reaction is related to RNA, not to enzyme. Assuming that all added RNA is active with respect to factor, it can be estimated from extrapolation of these curves that 1 molecule of HF₁ (mol wt 75,000) is required per molecule of Q₈ RNA.

Binding of Host Factor I to Single-stranded RNA—The association of HF₁ and Q₈ RNA was analyzed by zone sedimentation. Factor alone sediments at approximately 5 S, but when mixed with Q₈ RNA the active factor cosediments with the RNA (Fig. 5). Binding does not require the presence of other components required for RNA synthesis.
This binding is not limited to template RNA since HF1 also binds to E. coli rRNA and phage R23 and F2 RNA (Fig. 6). However, the reaction does appear to be specific for single stranded RNA. No binding was observed with double stranded reovirus RNA except for a faster sedimenting minor component which behaved as single stranded RNA. These experiments with nontemplate RNA suggest that factor binding is readily reversible, since factor activity could be measured in the Q/3 RNA-directed reaction. Approximately 75% of the factor activity was recovered. Binding was not detected with DNA, whether single stranded bacteriophage fd DNA, or double stranded PBSX or λ DNA.

Effect of Incubation of Q/3 RNA with Host Factor I—The possibility that HF1 causes an irreversible change in the template RNA was tested by incubating Q/3 RNA with the factor alone, with HF1 and HFII, or with HF1, HFII, and the Q/3 RNA polymerase. The RNA was then treated by phenol extraction to remove the added protein and tested for template activity in the presence and absence of HF1 (Table III). In every case HF1 was required for template activity, despite the previous treatment. This was true even when the RNA was first incubated with both factors, substrates (GTP, CTP, UTP), and polymerase, conditions which allow synthesis of a poly(G) sequence.4 This suggests that HF1 activity does not involve an irreversible alteration of RNA, such as a change in primary structure.

Effect of Host Factor I on Initiation of Synthesis—Successive steps in the synthesis of Q/3 RNA are the association of enzyme and Q/3 RNA, the initiation of synthesis, and chain elongation. As the nonspecific binding of RNA by the enzyme at 0°C and in the absence of substrates does not require the presence of HF1 (3, 24), it can be speculated that factor is required for a subsequent step leading to the initiation of synthesis or to chain elongation. An analysis of the requirement for HF1 in the initiation of synthesis has been performed by studies of pyrophosphate exchange, synthesis of a 5'-terminal polynucleotide, and [γ-32P]-GTP incorporation.

The pyrophosphate exchange reaction is completely dependent on HF1 (Fig. 9) suggesting that the factor is required for chain initiation. Since it has not been proven, however, that the pyrophosphate exchange reaction can discriminate between chain initiation and elongation, other more direct assays were carried out.

Synthesis of a limited sequence at the 5' terminus of the complementary strand can be analyzed by adding only ATP and GTP as substrates for the reaction since the first 14 nucleotides contain only guanylate and adenylyl residues (25). After incubation in the presence or absence of HF1, the reaction mixtures were cooled on ice and passed through Sephadex columns to separate the enzyme-template-nascent RNA complexes from the labeled substrates. Approximately 3.5 pmole of [3H]GMP were incorporated into the oligonucleotide product in the presence of HF1, while less than 0.1 pmole was incorporated when the factor was omitted (Fig. 9). It is clear from this experiment that HF1 is required for the synthesis of the initiating oligonucleotide of the complementary strand.

A more specific assay for the initiation of synthesis is the incorporation of [γ-32P]GTP into the 5' position of the complementary strand. This assay is limited to the presence of HF1 and HFII, and has been used extensively.

### Table III

| Components incubated with Q/3 RNA | Template activity |
|----------------------------------|------------------|
|                                 | Minus HF1 | Plus HF1 |
| none                             | 0.03      | 0.20     |
| HF1                              | 0.03      | 0.24     |
| HF1, HFII                        | 0.03      | 0.28     |
| HF1, HFII, Qp polymerase         | 0.02      | 0.20     |

*W. S. Hayward and P. Trow, unpublished observations.*
FIG. 8. Analysis of the requirement for HF₁ in the pyrophosphate exchange reaction. The reaction conditions are described under "Methods" and HF₁ was added as indicated.

FIG. 9. Synthesis of the 5' terminal oligonucleotide in the presence or absence of HF₁. Reaction mixtures containing only [α32P]GTP and ATP as substrates were incubated for 20 min at 37°. Samples were then cooled on ice and passed through Sephadex columns to separate the enzyme-template-nascent RNA complex from the labeled substrate as described under "Methods." Elution patterns for reaction mixtures incubated in the presence (●) or absence (○) of HF₁ are superimposed.

strand. As was previously shown, only GTP is incorporated at the 5' terminus and the direction of synthesis is from the 5' to 3' terminus (14). As described under "Methods," reaction mixtures containing only ATP and [γ-32P]GTP as substrates were incubated for 20 min in the presence or absence of HF₁. The [γ-32P]GTP was then diluted 50-fold with unlabeled GTP, and the appropriate components of the complete reaction mixture were added to both tubes. Incubation was continued for an additional 60 s to allow chain elongation. Whereas RNA synthesis during the chase was comparable in both reactions (150 and 177 pmol of [γ-32P]GTP incorporated), [γ-32P]GTP incorporation was observed only in the reaction mixture that contained HF₁ during the [γ-32P]GTP labeling period (0.23 and 0.01 pmol of [γ-32P]-GTP incorporated in the presence and absence, respectively, of HF₁). This suggests that neither phosphodiester bond formation nor irreversible binding of 5' terminal GTP occurs in the absence of factor I.

DISCUSSION

Several lines of evidence suggest that the role of HF₁ in the Q₁₅ RNA polymerase reaction is related specifically to the use of Q₃ RNA as template. As previously reported, factors are not required with any other template for the enzyme (3). Moreover, the factor appears to act directly on the RNA, rather than functioning as an enzyme subunit, since the concentration of HF₁ required in the reaction is stoichiometric with the concentration of RNA, but is independent of enzyme concentration. The factor binds to RNA even in the absence of enzyme or other components of the reaction, and this binding appears to be specific for single stranded RNA since it did not occur with double stranded reovirus RNA or with DNA. This observation suggests that HF₁ acts as a positive control element regulating the utilization of template in the reaction. This may be related to the synthesis in the reaction predominantly of Q₆ RNA, not the complementary strand (3, 26, 27). At this time the only known difference in the utilization of Q₆ RNA or its complement as template is the requirement for factors with Q₆ RNA but not with the complement (3).

HF₁ appears to act at an early step in the Q₁₅ polymerase reaction. The factor is absolutely required for pyrophosphate exchange, chain initiation (incorporation of [γ-32P]GTP), and polymerization when Q₆ RNA is template. Thus it seems likely that the factor acts at some step prior to the initiation of synthesis. The apparent first step in the polymerase reaction is the binding of RNA by the enzyme. This reaction takes place in the absence of factors, but occurs at multiple sites on nontemplate as well as template RNA and is readily reversible. It has recently been found, however, that if the enzyme-RNA complex is incubated with the two host factors and GTP, an irreversible complex is formed at a single site on template RNA (28). It is thus speculated that the factors are involved in the formation of a specific initiation complex of enzyme, RNA, factors, and GTP.

Q₆ RNA is known to contain extensive regions of hydrogen-bonded duplex structures (29). One possible model for HF₁ action is that the factor mediates a change in the secondary or tertiary structure of the RNA which allows the enzyme to bind to a site that is unavailable in the folded RNA molecule. Since only 1 molecule of HF₁ per molecule of RNA is required for synthesis, such an interaction would appear to involve only limited regions of the RNA, presumably at or near the enzyme-binding site. Perhaps because of this, attempts to demonstrate a change in secondary structure of Q₆ RNA after addition of HF₁ have been unsuccessful.⁴ HF₁ does not appear to change the primary structure of Q₆ RNA. Incubation of the Q₆ RNA and factor under

⁴ M. T. Franze de Fernandez, unpublished results.
a variety of conditions did not alter the RNA such that it could serve as template in the absence of HF. No RNase activity could be detected either by a change in sedimentation properties of the RNA after incubation with factor (see Fig. 5) or by a change in its infectivity. 6 In addition we could not detect either E. coli RNase II activity,7 phosphatase, 8 or adenylyl (cytidylyl) pyrophosphorylase 9 activity with the pure factor. Also, in collaboration with Dr. Maxine Singer, we tested pure RNase II for factor activity and did not detect any change in template activity or factor requirement 7 after treatment of Qp RNA with this enzyme. A further possibility is that HF acts in conjunction with HF 11 which also is required specifically with QP RNA as template. In all of our studies of the Qp RNA polymerase, with QP RNA as template, we have found both HF 1 and HF 11 to be required. As is described elsewhere (30), HF 11 also appears to act by binding to QP RNA. However, HF 11 differs from HF 1 in that more than 1 molecule is required per RNA chain. Moreover, it has been found that the HF 11 requirement is satisfied by a number of acid-soluble basic proteins of E. coli, as well as by protamine or calf thymus histones. These observations suggest that its mode of action is less specific than that of HF 1.

The role of HF 1 in the bacterial host is completely unknown. It can be distinguished from the other proteins known to be involved in QP RNA synthesis in E. coli by virtue of its molecular weight. Those include the RNA polymerase and its α subunit (31), the lac repressor (32), the ρ factor (33), and the host proteins associated with QP RNA polymerase activity (4, 5). Nevertheless, its specific binding to single stranded RNA and its role in regulating QP RNA replication suggests that HF 1 may be involved in RNA metabolism in E. coli.

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REFERENCES
1. FRANZ DE FERNANDEZ, M. T., EOTANG, L., AND AUGUST, J. T. (1968) Nature, 219, 588.
2. Shapiro, L., FRAZ Il DE FERNANDEZ, M. T., AND AUGUST, J. T. (1968) Nature, 220, 478.
3. August, J. T., Banerjee, A. K., Eotang, L., FRANZ DE FERNANDEZ, M. T., HORI, K., Kuo, C. H., Rensing, U., AND Shapiro, T. (1968) Cold Spring Harbor Symp Quant Biol., 33, 73.
4. KAMEN, R. (1970) Nature, 228, 527.

6 M. T. Franze de Fernandez and L. Shapiro, unpublished observations.
7 J. T. August, unpublished observations.
8 C. Prives, unpublished observations.
9 E. T. August, unpublished observations.
10 Kondo, M., Galli, R., AND WEISSMANN, C. (1970) Nature, 228, 525.
11 Eotang, L., AND AUGUST, J. T. (1968) Methods Enzymol., 12B, 530.
12 Eotang, L., AND AUGUST, J. T. (1971) in G. L. CANTONI AND D. R. Davies (Editors), Procedures in nucleic acid research, Vol. II, p. 829, Harper and Row, New York.
13 Kuo, C. H. (1971) in G. L. CANTONI AND D. R. Davies (Editors), Procedures in nucleic acid research, Vol. II, p. 846, Harper and Row, New York.
14 August, J. T., Orttz, P. J., AND Hurlwitz, J. (1962) J. Biol. Chem., 237, 3789.
15 BeLamy, A. R., Shapiro, L., August, J. T., AND Joklik, W. K. (1967) J. Mol. Biol., 99, 1.
16 Maryin, D. A., AND SCHALLER, H. (1966) J. Mol. Biol., 15, 1.
17 Baldwin, R. L., Barland, P., Fritsch, A., Goldthwait, D. A., AND Jacob, F. (1966) J. Mol. Biol., 17, 343.
18 Nakamoto, K., Mudd, J. A., Mangan, J., Huang, W. M., Subrahmanyan, T. V., AND Marmur, J. (1968) J. Mol. Biol., 31, 413.
19 Banerjee, A. K., Kuo, C. H., AND August, J. T. (1969) J. Mol. Biol., 40, 445.
20 HENDERSON, R. B., AND FORLORN, N. B. (1967) Methods Enzymol., 12B, 105.
21 Lowry, O. H., ROSEBROUGH, N. J., Farr, A. L., AND RANDALL, R. J. (1951) J. Biol. Chem., 193, 265.
22 Hill, R. L., AND DELANEY, R. (1967) Methods Enzymol., 11, 347.
23 Abraham, P., NEYENS, D. J., ENGLISH, P. D., AND KORR, A. (1967) Carbohydr. Res., 5, 340.
24 Malek, J. V. (1966) in H. KABEL AND N. P. SALTMAN (Editors), Fundamental techniques in virology, p. 334, Academic Press, New York.
25 Shapiro, A. L., VINSELLA, E., AND MAIZEL, J. V. (1967) J. Biol. Chem., 242, 815.
26 Bray, G. A. (1960) Anal. Biochem., 1, 279.
27 Hayward, W. S., AND FRAZIL DE FERNANDEZ, M. T. (1971) in G. L. CANTONI AND D. R. DAVIES (Editors), Procedures in nucleic acid research, Vol. II, p. 840, Harper and Row, New York.
28 Banerjee, A. K., Rensing, U., AND August, J. T. (1969) J. Mol. Biol., 45, 181.
29 Silverman, P. M. (1971) Fed. Proc., 30, 1316.
30 Goodman, H. M., BILLETTE, M. A., HINDELY, J., AND WEISSMANN, C. (1970) Proc. Nat. Acad. Sci. U. S. A., 67, 921.
31 Spiegelman, S., Pace, N. R., Mills, D. R., LIVISON, R., EICHOM, T. S., TAYLOR, M. M., PETTERSON, R. L., AND BISHOP, D. H. L. (1968) Cold Spring Harbor Symp. Quant. Biol., 33, 101.
32 Weissmann, C., FEIX, G., AND SLOR, H. (1968) Cold Spring Harbor Symp. Quant. Biol., 33, 83.
33 Silverman, P. M., AND August, J. T. (1970) Fed. Proc., 29, 340.
34 Billette, M. A., DADDEBERG, J. E., GOODMAN, H. M., HINDELY, J., AND WEISSMANN, C. (1969) Nature, 224, 1083.
35 Kuo, C. H., AND August, J. T. (1971) Fed. Proc., 30, 1316.
36 Dubnans, R. B. (1969) J. Biol. Chem., 244, 6158.
37 Gilbert, W., AND MÜLLER-HILL, B. (1970) in J. R. BECKWITH AND D. ZIPSER (Editors), The lactose operon, p. 93, Cold Spring Harbor Laboratory, New York.
38 Roberts, J. W. (1969) Nature, 224, 1168.
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