Initiation of transcription of favored templates by Qβ replicate requires a much lower GTP concentration than does transcription of templates that are selected against. Although the enzyme requires a high GTP concentration to initiate transcription of Qβ RNA, the presence of the host factor substantially reduces the GTP concentration requirement. In addition, the Qβ replicate preparation must contain ribosomal protein S1 for initiation to occur at low GTP. Mn²⁺ ions, which can substitute for host factor in Qβ RNA replication in vitro, and which reduce the template specificity of Qβ replicate, also reduce the GTP requirement for initiation. But while Mn²⁺ ions produce this effect with all templates, host factor is specific for Qβ RNA. When both host factor and Mn²⁺ are present, transcription of Qβ RNA occurs at a much lower GTP concentration. Thus, host factor and Mn²⁺ appear to reduce the GTP concentration requirement by different mechanisms.

Qβ replicate has long been noted for its template specificity (1). Nevertheless, this enzyme can transcribe a variety of synthetic polymers containing cytidylate and several heterologous natural RNA species in addition to Qβ RNA. All templates transcribed by Qβ replicate initiate with GTP (reviewed in Ref. 2). However, initiation of RNA synthesis requires widely different GTP concentrations, depending on the template (3). Mn²⁺, which increases the efficiency of transcription of RNAs normally selected against by Qβ replicate (4), reduces the GTP requirement with all templates. We have recently proposed that Qβ replicate template specificity is controlled by the template-determined concentration requirement for the initiating GTP (3). A prediction of this model is that transcription of homologous RNA would require substantially lower GTP concentrations than are required for heterologous natural RNAs.

There are five polypeptides required for Qβ RNA replication in vitro. Four of these, a phage-coded polypeptide and three polypeptides taken from the host protein synthetic machinery, make up Qβ replicate (2). Two of the latter, the elongation factors Tu and Ts, are required, along with the phage-coded polypeptide, for all RNA synthesis catalyzed by Qβ replicate. The other host-coded subunit of Qβ replicate, 30 S ribosomal protein S1, is required for transcription of Qβ RNA but not for heterologous RNA species (5). The fifth polypeptide, host factor, is a heat-stable, RNA-binding protein, also required for transcription of Qβ RNA only (6). Both S1 and HF² have been shown to be required for initiation of RNA synthesis and to bind to particular sequences on Qβ RNA (5-7).

In this communication, we show that in fact Qβ replicate alone requires a high GTP concentration in order to transcribe Qβ RNA. However, addition of HF greatly reduces the GTP requirement. We also show that this effect is specific for Qβ RNA, and that the presence of 30 S ribosomal protein S1 is necessary for host factor to reduce the GTP requirement.

EXPERIMENTAL PROCEDURES

Qβ replicate, purified as described (8), was approximately 95% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme contained equimolar amounts of the four subunits. It was found to incorporate nearly 1 pmol of [γ-³²P]GTP/pmol of enzyme present with poly(C) as template when aurintricarboxylic acid was added after initiation to prevent reinitiation. This enzyme was free of host factor activity as measured by stimulation of in vitro Qβ RNA replication after boiling the enzyme preparation. Replicase-lacking S1 was obtained from the leading edge of the Qβ replicase-containing peak from the final purification step (DEAE-Sephadex column) (9). It was as pure as the complete enzyme and was completely free of S1 as judged by gel electrophoresis. Qβ phase was purified by the procedure of Yamamoto et al. (10) with a second CsCl equilibrium centrifugation to ensure purity. Qβ RNA was extracted by the method of Overby et al. (11). It formed a single band on a 6 M urea-polyacrylamide gel. Poly(C) was purchased from P-L Biochemicals, f2 RNA, purified by the method of Nathans (12), was a gift of S. Kirtland (this laboratory).

Antibody to HF was a gift of G. Carmichael, Department of Pathology, Harvard Medical School, Boston, Mass. It was made against electrophoretically pure HF as described by Carmichael et al. (13). Two preparations of HF were used: One, a gift of G Carmichael, was made by poly(A) chromatography as described by Carmichael (14). The other, made by the procedure of Franze de Fernandez et al. (6) as modified by DuBow and Blumenthal (15), was a gift of M. S. DuBow, McGill University, Montreal, Canada. The two preparations were electrophoretically pure and had similar specific activities. S1, purified by the procedure of Carmichael (14), was electrophoretically pure and was a gift of G. Carmichael.

Qβ replicate assays were performed for 10 min at 30°C in the presence of 50 mM Tris-HCl, pH 7.5; 10 mM MgCl₂; 0.1 mM ATP, CTP, and [γ-³²P]UTP (100 nCi/mmol); and Qβ replicate, HF, GTP, and Qβ RNA at the concentrations given in the figure and table legends. Incorporation was linear for at least 40 min. After incubation, samples were precipitated with 5% trichloroacetic acid, filtered through 0.65-M Millipore DA nitrocellulose filters, dried, and counted in toluene/Omnifluor (New England Nuclear).

RESULTS

Effect of Host Factor and Mn²⁺—We have found previously that Mn²⁺, which reduces the template specificity of Qβ replicate, reduces the GTP requirement for initiation of tran-
cription of both heterologous natural RNAs and synthetic RNA polymers (3). Since Mn++ has previously been shown to allow in vitro Qβ RNA replication in the absence of host factor (4), we measured the GTP requirement for initiation of Qβ RNA replication in the presence and absence of Mn++, HF, or both. The data in Fig. 1 show that in the absence of Mn++ and HF, transcription of Qβ RNA requires high GTP concentrations. This curve is indistinguishable from that found when Qβ replicase transcribed f2 or 16 S rRNA (3). The addition of Mn++ ions results in a substantial reduction of the GTP requirement (Fig. 1). This result is similar to those obtained with heterologous natural RNAs (3). The presence of HF also leads to a substantial reduction of the GTP requirement (Fig. 1), a result predicted by the observation that Mn++ could substitute for HF in Qβ RNA replication in vitro. HF and Mn++ each stimulate total counts per min incorporated dramatically at low GTP concentration, but only stimulate slightly at high GTP. If both HF and Mn++ are present, replication proceeds at a much lower GTP concentration. When the experiment was repeated with twice the concentra-

![Graph](image1)

**Fig. 1.** Effect of HF and Mn++ on the GTP requirement for Qβ RNA transcription. Transcription of Qβ RNA (17 nM) by Qβ replicase (20 nM) was assayed for 10 min at 30°C as described under "Experimental Procedures," in the presence of: C, no additions; ○, 1 mM MnCl₂; □, 14 nM HF; and ■, both MnCl₂ and HF.

![Graph](image2)

**Fig. 2.** Effect of anti-HF on the GTP requirement for Qβ RNA transcription. Transcription of Qβ RNA with 14 nM HF was assayed as in the legend to Fig. 1 in the presence (x) or absence (○) of 50 μl/ml of purified anti-HF. The antibodies were preincubated with the HF in the complete reaction mixture (~GTP) for 10 min at 37°C before addition of Qβ replicase and GTP. The data are presented as a percentage of the maximal value obtained under each condition.

![Graph](image3)

**Fig. 3.** Effect of HF on transcription of f2 RNA and poly(I,C). f2 RNA at 25 μg/ml (x, y) and poly (I,C) at 50 μg/ml (z) were transcribed for 10 min at 30°C in the presence (x, y) or absence (z) of 14 μM HF as described under "Experimental Procedures." In the poly(I,C) reaction, the labeled NTP was 50 μM [3H]CTP at 100 mCi/mmol.

![Table](image4)

**Table 1.** Effect of glycerol on GTP requirement and total incorporation

<table>
<thead>
<tr>
<th>GTP requirement (50% maximal)</th>
<th>Incorporation (at optimum GTP)</th>
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<tbody>
<tr>
<td>GTP µM</td>
<td>Incorporation (at optimum GTP)</td>
</tr>
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<tr>
<td>Mn++</td>
<td>105</td>
</tr>
<tr>
<td>Mg++ + HF</td>
<td>90</td>
</tr>
<tr>
<td>Mn++ + HF</td>
<td>24</td>
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* ND, not determined.
tween Qβ RNA and HF must be involved in the reduction of the requirement for GTP.

Effect of Glycerol—We have previously shown that a high glycerol concentration allows efficient transcription of f2 RNA by Qβ replicase, but does not result in a reduction of the GTP requirement (3). The data of Table I demonstrate a similar effect with Qβ RNA. The presence of 25% glycerol results in a dramatic stimulation of Qβ replicase-dependent transcription of Qβ RNA in the absence of HF or Mn2+. However, the glycerol reduces the GTP optimum only slightly. Mn2+, on the other hand, reduces the GTP optimum in the presence or absence of glycerol. The mechanism by which glycerol works is not known. In any case, these data show that unlike Mn2+ and HF, glycerol dramatically stimulates Qβ RNA transcription in vitro without reducing the requirement for GTP. The data in Table I also demonstrate that HF reduces the GTP optimum and stimulates total synthesis in the presence of glycerol.

Requirement for S1—One of the subunits of Qβ replicase, S1, is required specifically for transcription of Qβ RNA, as is HF (5). When enzyme lacking S1 is tested for transcription of Qβ RNA, it is found to be active, but only at high GTP concentration (Fig. 4). The addition of S1 to the enzyme does not affect this synthesis. The enzymatic activity is stimulated by the presence of HF, but the requirement for GTP remains high. However, if both HF and S1 are added, then a significant reduction in the GTP requirement is observed. Thus, both HF and S1, the two proteins required specifically for Qβ RNA transcription, are required for reduction of the GTP requirement with the homologous template.

**DISCUSSION**

Qβ replicase shows a high degree of template specificity. Under most assay conditions, it will transcribe Qβ RNA and synthetic RNA molecules containing cytidylate, but not the RNA of several related single-stranded RNA bacteriophages (2). Qβ replicase initiates all RNA synthesis with GTP. The concentration of GTP required for initiation varies widely, depending on the RNA species being transcribed. The enzyme will transcribe a great variety of RNAs, but those RNAs which are normally selected against require a much higher GTP concentration for initiation than those more readily transcribed. We have hypothesized that the quality of the initiation site is determined by the nature of the interaction between Qβ replicase and each RNA species (9). Mn2+ ions have been shown to reduce the high template specificity of Qβ replicase (4). Furthermore, Mn2+ eliminates the high GTP concentration requirement for all templates (3). Hence, it seems likely that the mechanism by which Qβ replicase selects against heterologous templates involves creation of initiation complexes requiring a high GTP concentration for initiation.

Since Qβ RNA is the most favored template among natural RNA species, we might expect Qβ RNA transcription to have the lowest GTP concentration requirement, if the mechanism described above is actually used to discriminate among templates. However, we find that transcription of Qβ RNA by Qβ replicase requires as high a GTP concentration as do natural RNAs which are selected against as templates. This apparent paradox is resolved by the observation that Qβ replicase does not effectively transcribe Qβ RNA unless HF is present. Thus, the finding that HF reduces the GTP requirement for transcription of Qβ RNA provides support for the idea that the GTP concentration is an important factor in template selection by Qβ replicase.

Palmenberg and Kaeberg (4) have shown that the presence of Mn2+ obviates the need for HF for in vitro Qβ RNA replication. Similarly, we find that either HF or Mn2+ will reduce the GTP concentration requirement and to approximately the same extent. When both are present, a much larger reduction in the GTP concentration requirement is observed. This result indicates that HF and Mn2+ operate by different mechanisms. Mn2+ may act by forming a complex with GTP that binds more tightly than Mg2+GTP to the initiation site on Qβ replicase. Mn2+ thereby facilitates transcription of all RNA species. In contrast to the global effect of Mn2+ on initiation, HF is known to interact specifically with Qβ RNA. The interaction of HF with the RNA, rather than with the replicase, is required for in vitro Qβ RNA replication (6). From the results presented here, we suggest that HF (along with S1) acts by aligning Qβ RNA with Qβ replicase in such a way as to favor the formation of a binding site with a high affinity for GTP.

We do not yet know the mechanism by which HF interacts with Qβ RNA to effect this change. Perhaps it alters the RNA secondary structure by binding to a region of the RNA which normally base-pairs with another RNA site required for initiation. By analogy with the multiple base-pairing scheme hypothesized for attenuator regions (17), HF could thereby inactivate a preemining base sequence and "unmask" a sequence necessary for formation of a high affinity initiation site.

In uninfected bacteria, HF is found associated with the 30 S ribosomal subunit, but its role is unknown (18). Assuming that it performs a function in protein biosynthesis related to its function in Qβ RNA replication, we propose that HF could interact with a specific sequence on some mRNA species to facilitate initiation of translation. Based on our findings with Qβ replicase, we have recently hypothesized that "strong" ribosome-binding sites could result from formation of template-induced high affinity binding sites for fMet-tRNA (3). Consequently, HF might alter the mRNA secondary structure of a group of mRNAs containing the specific sequence to improve the quality of the translation initiation site. The fact that f2 replicase also requires a host factor but that this protein is apparently different from Qβ HF (16) suggests the possibility that HF is only one of a group of proteins that could recognize classes of mRNA initiation regions.

**Acknowledgments**—We thank B. Polisky for critical reading of the manuscript and B. Saari for technical assistance. S. Kucher, G. Carmichael and M. S. DuBow provided gifts of proteins.

**REFERENCES**

**FIG. 4. Effect of S1 and HF on Qβ RNA transcription by Qβ replicase lacking S1.** Transcription of Qβ RNA (17 nM) by Qβ replicase lacking S1 (21 nM) was assayed for 10 min at 30°C in the presence of: ○, no additions; ●, 71 nM S1; □, 14 nM HF; and ■, both S1 and HF.
Roles of Host Factor and S1 in Qβ RNA Replication