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Qβ replicase template specificity: Different templates require different GTP concentrations for initiation

(template choice/RNA phage/RNA synthesis/Mn^{2+})

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ABSTRACT **Qβ replicase is notable for its high degree of template specificity. It has been shown to transcribe Qβ RNA and synthetic polymers containing cytidylate. However, other natural RNAs are not transcribed unless Mn^{2+} ions are present. The enzyme initiates all RNA synthesis with GTP. In this report it is shown that Qβ replicase can transcribe heterologous natural RNA species in the absence of Mn^{2+} if sufficient GTP is present. Each RNA tested requires a different GTP concentration for initiation. These results indicate that the site for the initiating nucleoside triphosphate on Qβ replicase is strongly influenced by the template. It is proposed that the high degree of template specificity is a consequence of the fact that different templates induce initiation sites with varying affinities for GTP. Two lines of evidence support this idea. First, Mn^{2+} ions, which reduce template specificity, reduce the concentration of GTP required for initiation. Second, high ionic strength, which decreases transcription of all templates except Qβ RNA, increases the GTP requirement. The possibility is considered that variable promoter or ribosome binding site strengths could result from a mechanism similar to that proposed here.**

Qβ replicase has the ability to discriminate between Qβ RNA and most other natural RNA species, even those of the closely related group I RNA phages (1). In spite of years of study, the mechanism by which this high template specificity is achieved remains a mystery (see ref. 2 for review). In addition to Qβ RNA, the enzyme can replicate several different small RNA species found in Qβ-infected cells, the "6S" RNAs (3–7). Weissmann (6) has hypothesized that the 3′ end of the template is positioned correctly with respect to internally bound Qβ replicase only with Qβ and 6S RNAs and, thus, all other RNA species are selected against.

Qβ replicase is also able to transcribe random-sequence polymers that contain cytidylate residues (8). With these templates, as with all others transcribed by this enzyme, GTP is the initiating nucleoside triphosphate (2). There are several important differences between the Qβ RNA replication and synthetic template transcription reactions (reviewed in ref. 2). The latter reaction requires only three of the five polypeptides involved in RNA replication (9); it is inhibited by high ionic strength (10); and it is less sensitive to antibodies made against the host-coded subunits of Qβ replicase (11).

Recently several methods of overcoming the template specificity of Qβ replicase have been reported. These methods have shed some light on the processes involved in template selection. The template specificity can be overcome by the addition of a primer, a short oligonucleotide capable of forming hydrogen bonds with the RNA to be transcribed (12). Apparently any RNA can be recognized by using this technique. These results suggest that the template specificity is a function of successful initiation, rather than template binding, because the primer presumably bypasses only the initiation reaction.

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This conclusion is further supported by the finding that template specificity is significantly reduced when Mn^{2+} ions are present (13, 14). This effect must also represent increased initiation, rather than template binding, because Qβ replicase can bind tightly to a wide variety of RNAs in the absence of divalent cations (15). How does Mn^{2+} enable the enzyme to initiate on templates to which it will normally bind but which it will not transcribe?

The experiments reported here indicate that the high template specificity of Qβ replicase may be a result of the requirement for high levels of GTP for transcription of restricted templates. Different templates require different GTP concentrations for initiation. Mn^{2+} decreases the amount of GTP required, whereas high ionic strength, which increases template specificity, increases the GTP requirement.

MATERIALS AND METHODS

Qβ replicase, purified as described (16), was approximately 95% pure, as judged by sodium dodecyl sulfate gel electrophoresis. The enzyme contained equimolar amounts of the four subunits. It incorporated nearly 1 pmol of [γ-^{32}P]GTP per pmol of enzyme present with poly(C) as template when aurintricarboxylic acid was added after initiation to prevent reinitiation.

f2 RNA, purified by the method of Nathans (17), was a gift from M. Beremand (this laboratory). 16S rRNA was purified by the method of Traub *et al.* (18) and was a gift from S. Kirtland (this laboratory). Both RNA preparations contained approximately 25% fragmented molecules, as judged by agarose/polyacrylamide gel electrophoresis.

All Qβ replicase assays were for 10 min at 30°C in the presence of 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, and 4.2 μg of Qβ replicase per ml. Synthetic templates were present at 50 μg/ml, whereas f2 and 16S RNA were at 20 μg/ml. When synthetic templates were assayed, the labeled NTPs were present at 50 μM. All NTPs (except GTP) were at 100 μM for transcription of natural RNAs. Labeled NTPs were present at 5 μCi/ml (1 Ci = 3.7×10^{10} becquerels) with synthetic templates and 10 μCi/ml with natural RNAs. All reactions were initiated by the addition of GTP at the indicated concentrations. After incubation, samples were precipitated with 5% trichloroacetic acid, filtered through 0.65-μm Millipore DA nitrocellulose filters, and dried, and radioactivity was measured in toluene/Omnifluor (New England Nuclear).

RESULTS

Template Comparison. Qβ replicase has been reported to have a K_m for initiation of 12–20 μM GTP for the poly(C) reaction (19, 20) and of 200 μM GTP for the Qβ RNA replication reaction (19). This unexplained difference led me to attempt to determine the K_m for GTP with a group of other templates.

Abbreviation: EF-Tu, elongation factor Tu.

I measured total RNA synthesis at 30°C for 10 min with three synthetic random-sequence copolymers containing cytidylate residues and two heterologous natural RNAs at GTP concentrations ranging from 5 μM to 1.5 mM. Reciprocal plots of the data did not produce straight lines (not shown). This could be due to the complex interactions between Qβ replicase and guanine nucleotides: the presence of the GTP-binding site on one of the subunits, elongation factor Tu (EF-Tu), causes the enzyme to be inactivated by GTP (20). It is also possible that the complex kinetics are a result of the fact that the templates are either synthetic RNAs with random 3′-terminal sequences or partially degraded natural RNA molecules containing a mixture of 3′-terminal sequences. It is shown below that the GTP requirement is different with different templates. Thus the mixture of 3′-terminal sequences present in each RNA preparation might result in a mixture of enzyme–template complexes with different K_m values for GTP. In any case, in order to facilitate template comparisons the data have been calculated as a percentage of the maximal value obtained with each template. The results clearly show that maximal synthesis occurs at widely different GTP concentrations, depending on the template (Fig. 1A). Relatively high GTP is required for poly(I,C) compared to poly(U,C) and poly(A,C). Furthermore, still higher GTP is necessary for transcription of f2 or 16S rRNA preparations.

The RNA phage replicases require much higher levels of GTP than of the other nucleoside triphosphates (ref. 10 and unpublished data). Because these enzymes always initiate with GTP, the variation in the GTP requirement seen in Fig. 1 most likely results from a variation in the initiation requirement. Two experiments were performed to demonstrate that this is so. First, the GTP concentration experiment shown in Fig. 1A was repeated [with poly(I,C)] in the presence and absence of 0.2 mM ITP. Because ITP can replace GTP for elongation of preinitiated polynucleotide chains but not for the initiation reaction (12), a high level of ITP would increase synthesis only if the elongation reaction is limited by the GTP concentration. However, the ITP had no effect (data not shown). Hence, the low GTP concentration must be limiting the initiation reaction. In the second experiment, the normal initiation reaction is bypassed by the use of a primer, GpG, to initiate synthesis. In this case the prediction is that the presence of GpG would increase synthesis only if low GTP limits the initiation reaction because GpG cannot be inserted internally in a growing RNA chain. In fact, the primer allowed high levels of synthesis with all of the templates at much lower GTP concentrations than those found to be limiting in Fig. 1A (unpublished experiments and Fig. 3A). Hence, the variable level of GTP necessary for transcription by Qβ replicase, specified by the template, is a requirement of the initiation reaction.

Effect of Mn^{2+}. The above results suggested the possibility that the observed template-dependent variation in the requirement for the initiating nucleoside triphosphate might result in the high template specificity of Qβ replicase. To examine this possibility further, I measured the effect of Mn^{2+} ions on the GTP concentration curves, because Mn^{2+} has been shown to extend the range of templates transcribed by Qβ replicase (13, 14). Fig. 1B shows that Mn^{2+} dramatically lowers the GTP requirement with all five templates tested. The fact that the differences between the templates were maintained in the presence of Mn^{2+} was expected; Palmenberg and Kaesberg (13) found large differences among natural RNAs in the efficiency of Mn^{2+}-dependent transcription.

The curve shown in Fig. 2 indicates that, as the $MnCl_2$ concentration is increased, the amount of poly(I,C) transcription at 10 μM GTP is increased until approximately 0.6 mM $MnCl_2$. The Mn^{2+} optimum for transcription of heterologous templates is also in this range (13). Thus the correlation between Mn^{2+}-dependent alterations of template specificity and GTP requirement is strengthened. Nevertheless, the data do not prove that the alteration by Mn^{2+} in the GTP requirement for initiation is the *cause* of the alteration in the template specificity.

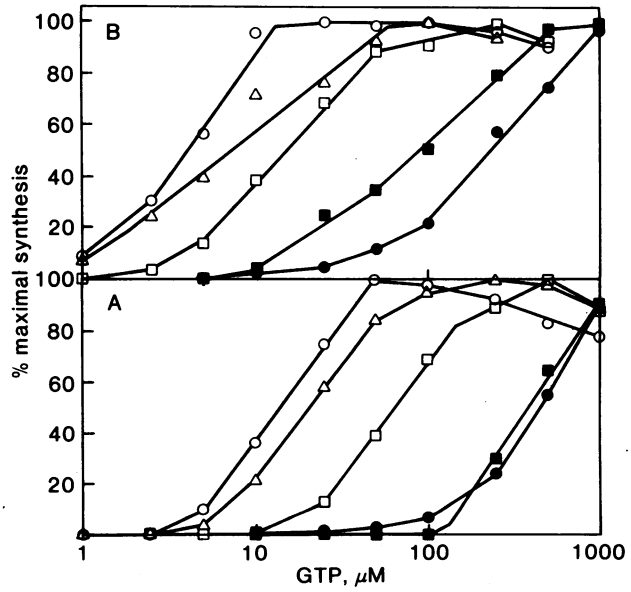

FIG. 1. Effect of GTP concentration on transcription of natural and synthetic RNAs in the absence (*A*) or presence (*B*) of 1 mM $MnCl_2$. f2 and rRNA were transcribed in the presence of 25% glycerol. Data are presented as percent of the cpm incorporated at the optimal GTP concentration. (*A*) These values were (cpm): ○, poly(U,C), 11,762; △, poly(A,C), 2879; □, poly(I,C), 2527; ●, f2 RNA, 2575; ■, 16S rRNA, 353. (*B*) The values were (cpm): ○, poly(U,C), 17,983; △, poly(A,C), 4264; □, poly(I,C), 7044; ●, f2 RNA, 8781; ■, 16S rRNA, 950.

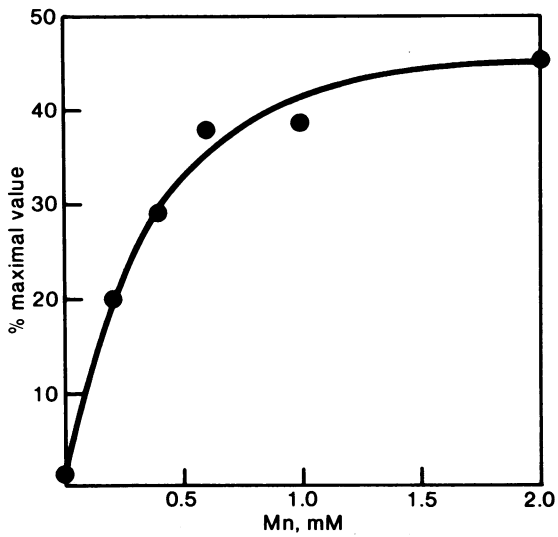

FIG. 2. Effect of Mn^{2+} concentration on the GTP requirement for transcription of poly(I,C). Transcription of poly(I,C) in the presence of 10 μM GTP and the indicated concentrations of $MnCl_2$ was measured. The data are presented as a percent of the value determined at the optimal GTP concentration (given in parentheses) for each $MnCl_2$ concentration. These values were (cpm): 0 M, 2527 (500 μM); 0.2 mM, 9239 (250 μM); 0.4 mM, 9350 (250 μM); 0.6 mM, 10,498 (100 μM); 1 mM, 7044 (250 μM); 2 mM, 11,535 (100 μM).

It could be argued that the template- and Mn^{2+}-dependent variations in the GTP requirement for initiation might be a result of synthesis by subfractions of the Qβ replicase preparations that are active under different conditions and that vary in their K_m values for GTP. In order to eliminate this possibility, I performed [γ-^{32}P]GTP labeling experiments with the three synthetic polymers to obtain estimates of the fraction of the enzyme initiating with each polymer (at saturating GTP concentration). The values obtained ranged from 0.45 pmol initiated per pmol of Qβ replicase for poly(A,C) in the Mg^{2+}-dependent reaction to 1.15 pmol for poly(U,C) in the presence of Mn^{2+}. Because the variation in this parameter is small and because a relatively large fraction of the enzyme is initiating, it is fair to conclude that the template- and Mn^{2+}-induced variations in the GTP requirement are due to variation in the whole population of enzyme molecules, at least with the synthetic polymers. We also obtained estimates of the product chain lengths from this experiment. These values ranged from 130 nucleotides per polynucleotide chain for poly(I,C) to 470 for poly(U,C). The presence of Mn^{2+} did not significantly alter the chain length (data not shown).

Effect of Ionic Strength. In order to extend the correlation between initiation specificity and GTP concentration, I measured the effect of another parameter that affects template specificity: ionic strength. Whereas the poly(C)-dependent reaction is strongly inhibited by salt, the homologous RNA replication reactions of both Qβ and f2 replicases have relatively high salt optima (≈0.15 M NaCl) (refs. 10 and 21 and unpublished observations). Furthermore, the transcription of 6S RNAs (found in Qβ-infected cells) by Qβ replicase is very sensitive to increase in ionic strength under conditions that allow extensive Qβ RNA replication (5, 9). Hence, high ionic strength can be considered to increase the template specificity of the enzyme, and I would predict, therefore, that it might increase the amount of GTP required for initiation. The data in Fig. 3 demonstrate that, in fact, the GTP requirement for transcription of poly(I,C) increases with increasing ionic strength, both in the absence (Fig. 3*A*) and in the presence (Fig. 3*B*) of Mn^{2+}.

These data are replotted as a function of salt concentration in Fig. 4. This plot demonstrates that salt is most inhibitory at low GTP concentrations. The previously observed inhibition of Qβ replicase by high ionic strength (21) must result from the increased requirement for GTP because at high GTP concentration the reaction is not inhibited even by substantial salt concentrations. It can also be seen that at a given GTP concentration, a much lower salt concentration is required to inhibit the reaction in the absence of Mn^{2+} than in its presence.

Effect of Glycerol on Heterologous Template Transcription. During this work I discovered that glycerol stimulates *in vitro* transcription of natural RNAs by Qβ replicase. The maximal effect is seen at 25% glycerol (data not shown). This stimulation does not result from a reduction in the GTP requirement. The data in Fig. 5 show that both in the absence (open symbols) and the presence (filled symbols) of Mn^{2+}, the GTP required for maximal synthesis (Fig. 5*B*) is not altered appreciably by the presence of glycerol, although the level of synthesis is dramatically stimulated by glycerol at all GTP concentrations in the Mg^{2+}-dependent reaction (Fig. 5*A*). Thus, although both Mn^{2+} and salt act by altering the concentration of GTP required for initiation, glycerol stimulates transcription of heterologous RNAs by another, as yet unknown, mechanism.

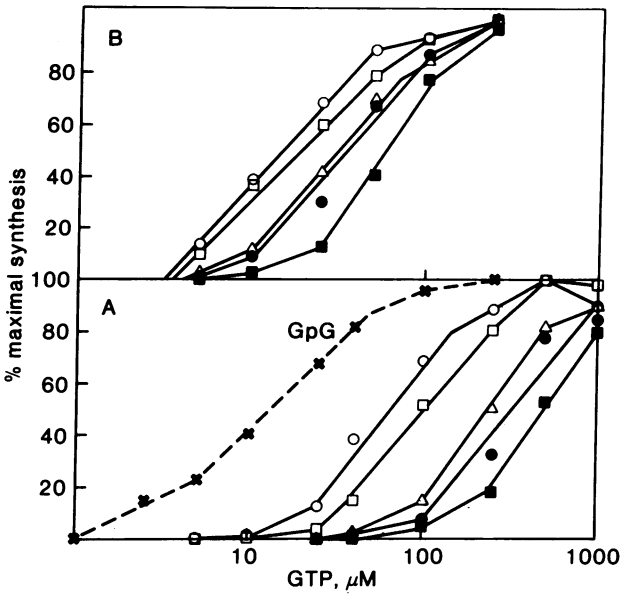

FIG. 3. Effect of salt concentration on the GTP requirement for transcription of poly(I,C) in the absence (*A*) or presence (*B*) of 2 mM $MnCl_2$. Data are presented as a percent of the value obtained at the optimal GTP concentration for each salt concentration. (*A*) These values were (cpm): ○, no salt, 2527; □, 75 mM NaCl, 3455; △, 150 mM NaCl, 2886; ●, 225 mM NaCl, 3768; ■, 300 mM NaCl, 1596; x – – x, no salt plus 0.2 mM GpG, 4371. (*B*) The values were (cpm): ○, no salt, 7044; □, 75 mM NaCl, 8802; △, 150 mM NaCl, 9249; ●, 225 mM NaCl, 7021; ■, 300 mM NaCl, 5145.

DISCUSSION

The findings reported in this paper suggest a relationship between template specificity and the concentration of GTP required for initiation of RNA synthesis. First, different templates require different GTP concentrations for maximal transcription. Second, Mn^{2+} ions, which have been shown to extend the range of templates used by Qβ replicase (13, 14), decrease the GTP requirement. The Mn^{2+} concentration required for minimizing the GTP level necessary for initiation (Fig. 2) is similar to that required for maximizing transcription of heterologous RNAs (13). Third, high ionic strength, which has been shown to select against transcription of RNA molecules other than Qβ (5, 9, 10, 21), is shown here to increase the GTP requirement for initiation on these templates.

If the template-determined GTP concentration requirement is an important factor in template choice by Qβ replicase, then transcription of Qβ RNA should require less GTP than other natural RNAs. Unfortunately, we cannot directly test this prediction because of possible complications arising from the requirement for host factor, which performs an unknown (but RNA-specific) function in Qβ RNA replication (2). In a preliminary experiment I found that the Qβ replicase used in these studies, which does contain some host factor, requires about as much GTP as does poly(I,C). Thus, Qβ RNA appears to require substantially less GTP than do f2 RNA or rRNA, at least in the presence of subsaturating levels of host factor.

However, it is unlikely that Qβ replicase selects against heterologous RNAs solely by a mechanism involving GTP insufficiency. Previous findings have suggested that other factors are involved. First, replicase lacking ribosomal protein S1 loses the ability to transcribe Qβ RNA but not heterologous RNA molecules (9). This shows that S1 is probably directly involved in site selection on Qβ RNA. Second, internal RNA sites have been implicated in recognition of Qβ RNA (6, 7) and 6S RNAs (4, 5) by Qβ replicase. Weissmann (6) has hypothesized that only these RNAs have the proper secondary structure such that

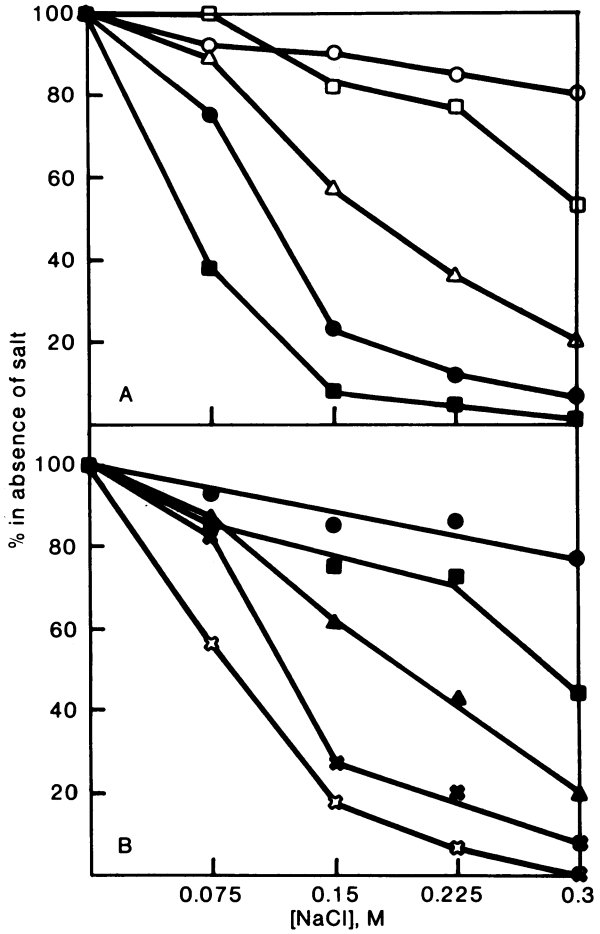

FIG. 4. Effect of GTP concentration on salt inhibition of poly(I,C) transcription in the absence (*A*) or presence (*B*) of 2 mM $MnCl_2$. The data of Fig. 3 were recalculated as a percent of the values obtained in the absence of salt for each GTP concentration: ⊠, 5 μM; X, 10 μM; ▲, 25 μM; ■, 50 μM; ●, 100 μM; △, 250 μM; □, 500 μM, and ○, 1 mM.

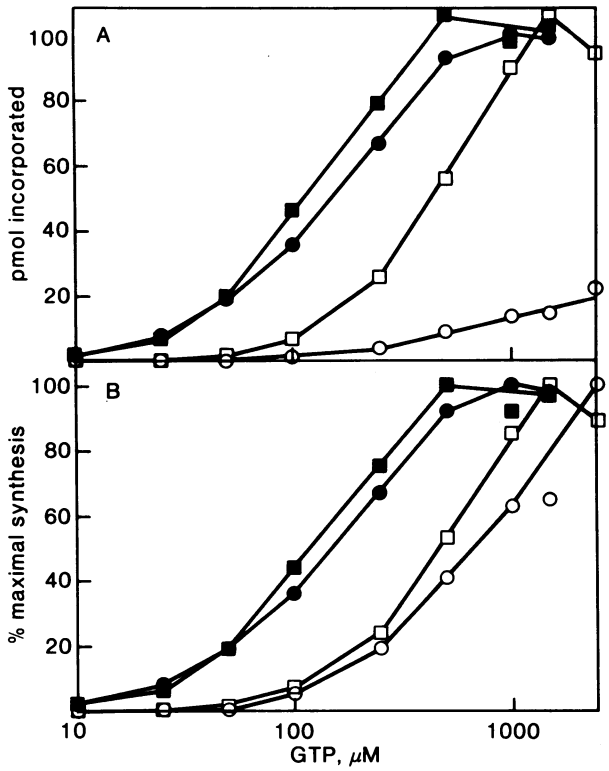

FIG. 5. Effect of Mn^{2+} and glycerol on the GTP requirement for f2 RNA transcription. ○, No additions; □, 25% glycerol; ●, 1 mM $MnCl_2$; ■, 25% glycerol plus 1 mM $MnCl_2$. *B* shows the data of *A* as a percentage of the maximal value achieved for each assay condition.

the 3′ end of the molecule is positioned properly with respect to the internally bound enzyme. Finally, in the absence of GTP or divalent cation, Qβ replicase binds approximately 10-fold more tightly to Qβ RNA than it does to heterologous RNAs (15).

Our data suggest that the limited transcription of heterologous RNA species allowed by Mn^{2+} (13, 14) may be accomplished through lowering of the GTP requirement for initiation and that the increased template specificity afforded by high salt conditions may result from the increased GTP requirement. How might ionic strength and Mn^{2+} ions influence the level of GTP required for initiation? One possibility is that they do so through alterations in the enzyme structure. We have previously found that high ionic strength results in a "tighter" association between EF-Tu·Ts and the other Qβ replicase subunits (21, 22). The site on the enzyme that binds GTP for initiation might be less accessible in the high-ionic-strength configuration. In fact, the GTP-binding site on EF-Tu in Qβ replicase does bind less GTP as the ionic strength is increased (23), but there is no evidence that this site is involved in initiation (2, 21, 23).

The general hypothesis that alterations in enzyme structure might directly alter template specificity is supported by the recent isolation of an altered Qβ replicase containing kirromycin-resistant mutant EF-Tu (24). This enzyme is unstable in the reaction mixture and also shows a more stringent template specificity than wild-type Qβ replicase. It will transcribe Qβ RNA but will only transcribe poly(C) if Mn^{2+} is present (24).

Mn^{2+} might decrease the GTP requirement by increasing the accessibility of the initiation site, perhaps also by changing the enzyme quaternary structure. However, when trypsin cleavage was used as a conformational probe (25), I could not detect any structural alteration caused by Mn^{2+}. [The alteration caused by high ionic strength is easily demonstrated by this technique (unpublished observations).] Alternatively, Mn^{2+} might lower the GTP requirement directly by complexing with the GTP in place of Mg^{2+}. The Mn·GTP complex could bind more tightly to the initiation site on Qβ replicase than does Mg·GTP.

How does the template influence the level of GTP required for initiation? The initiation site is presumably made up of components of both enzyme and template. It is possible that the base sequence at the enzyme–template interface directly influences the tightness of GTP binding or, alternatively, that each particular template causes a change in enzyme structure such that the GTP is bound more or less tightly. If either of these explanations is correct, it means the RNA to be copied directly influences the enzyme such that it has a greater or lesser probability of transcribing that RNA. In the case of Qβ replicase, this mechanism would result in selection against transcription of heterologous RNA molecules.

It is possible that a similar mechanism could be involved in determination of promoter and ribosome binding site strengths. That is, the sequence of bases in the promoter or the mRNA could influence the structure of the RNA polymerase or ribosome such that the initiating nucleoside triphosphate or fMet-tRNA (respectively) is bound more or less tightly. RNA polymerase does require high levels of the initiating nucleoside

triphosphate (26), but it is not clear whether the "closed" to "open" complex transition or the binding of the first nucleoside triphosphate is the rate-limiting step under *in vivo*-like conditions (26–28). The possibility that different promoters induce different K_m values of initiation has never been systematically tested. If the model is correct, promoter strength should correlate with the level of nucleoside triphosphate required for transcription initiation. In the case of initiation of protein synthesis, the model predicts that the presence of the mRNA would influence the tightness of binding of the fMet-tRNA, and that the K_m for fMet-tRNA binding should be lower with more highly favored ribosome binding sites. This prediction has also not been tested. It should be pointed out that the model provides a sensitive mechanism by which the cell could globally regulate classes of genes in response to change in the levels of initiating nucleoside triphosphates or fMet-tRNA.

Note Added in Proof. We have recently found that transcription of Qβ RNA by host factor-free Qβ replicase requires high GTP concentration for initiation. The addition of host factor substantially reduces the GTP requirement. This effect of host factor is specific to transcription of Qβ RNA and requires the presence of the S1 subunit of Qβ replicase.

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1. Haruna, I. & Spiegelman, S. (1965) *Proc. Natl. Acad. Sci. USA* **54,** 579–587.
2. Blumenthal, T. & Carmichael, G. G. (1979) *Annu. Rev. Biochem.* **48,** 525–548.
3. Banerjee, A. K., Rensing, U. & August, J. T. (1969) *J. Mol. Biol.* **45,** 181–193.
4. Schaffner, W., Ruegg, K. J. & Weissmann, C. (1977) *J. Mol. Biol.* **117,** 877–907.
5. Mills, D. R., Nishihara, T., Dobkin, C., Kramer, F. R., Cole, P. E. & Spiegelman, S. (1977) in *Nucleic Acid-Protein Recognition,* ed. Vogel, H. J. (Academic, New York), pp. 533–547.
6. Weissmann, C. (1974) *FEBS Lett.* **40,** S10–S18.
7. Vollenweider, H. J., Koller, T., Weber, H. & Weissmann, C. (1976) *J. Mol. Biol.* **101,** 367–377.
8. Hori, K., Eoyang, L., Banerjee, A. K. & August, J. T. (1967) *Proc. Natl. Acad. Sci. USA* **57,** 1790–1797.
9. Kamen, R., Kondo, M., Romer, W. & Weissmann, C. (1972) *Eur. J. Biochem.* **31,** 44–51.
10. Fedoroff, N. V. & Zinder, N. D. (1972) *J. Biol. Chem.* **247,** 4586–4592.
11. Carmichael, G. G., Landers, T. A. & Weber, K. (1976) *J. Biol. Chem.* **251,** 2744–2748.
12. Feix, G. & Hake, H. (1975) *Biochem. Biophys. Res. Commun.* **65,** 503–509.
13. Palmenberg, A. & Kaesberg, P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1371–1375.
14. Haruna, I. & Spiegelman, S. (1965) *Proc. Natl. Acad. Sci. USA* **54,** 1189–1193.
15. Silverman, P. M. (1973) *Arch. Biochem. Biophys.* **157,** 222–233.
16. Blumenthal, T. (1979) *Methods Enzymol.* **60,** 628–638.
17. Nathans, D. (1968) *Methods Enzymol.* **12B,** 787–791.
18. Traub, P., Mizushima, S., Lowry, C. V. & Nomura, M. (1971) *Methods Enzymol.* **20,** 391–407.
19. Mitsunari, Y. & Hori, K. (1973) *J. Biochem.* **74,** 263–271.
20. Blumenthal, T. (1977) *Biochim. Biophys. Acta* **478,** 201–208.
21. Blumenthal, T., Young, R. A. & Brown, S. (1976) *J. Biol. Chem.* **251,** 2740–2743.
22. Young, R. A. & Blumenthal, T. (1975) *J. Biol. Chem.* **250,** 1829–1832.
23. Landers, T. A., Blumenthal, T. & Weber, K. (1974) *J. Biol. Chem.* **249,** 5801–5808.
24. Blumenthal, T., Saari, B., Van der Meide, P. H. & Bosch, L. (1980) *J. Biol. Chem.*, in press.
25. Douglass, J. & Blumenthal, T. (1979) *J. Biol. Chem.* **254,** 5383–5387.
26. McClure, W. R., Cech, C. L. & Johnston, D. E. (1978) *J. Biol. Chem.* **253,** 8941–8948.
27. Rhodes, G. & Chamberlin, M. J. (1975) *J. Biol. Chem.* **250,** 9112–9120.
28. Stahl, S. J. & Chamberlin, M. J. (1977) *J. Mol. Biol.* **112,** 577–601.