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Characterization of the Initial Peptide of Qβ RNA Polymerase and Control of Its Synthesis

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ABSTRACT **Bacteriophage Qβ RNA directs the cell-free synthesis of two fMet-containing dipeptides prior to the first round of ribosomal translocation. One of these, fMet-Ala, corresponds to the initial segment of the Qβ coat protein. In the present work we take advantage of the fact that translation of the Qβ RNA polymerase cistron is repressed by its coat protein to correlate the other peptide, fMet-Ser, with the Qβ RNA polymerase gene. Our experiments show that repression affects translation of the first two codons of the polymerase cistron, thereby isolating the effect of Qβ coat repressor. Further studies, using single rounds of translocation of the Qβ mRNA and codon-specific tRNAs, allow us to predict the first three amino acids of the Qβ RNA polymerase protein, fMet-Ser-Lys, and to suggest that the initiation region of the Qβ RNA polymerase cistron has the sequence** $\cdots$AUG·UC_C^U·$AA_G^A\cdots$

Using a highly purified cell-free system from *Escherichia coli* in which translocation of viral message is prevented, we have shown that phage Qβ RNA directs the synthesis of only two fMet-containing dipeptides (1). One of these, fMet-Ala, corresponds to the expected Qβ coat dipeptide (2). In the absence of well-characterized phage proteins or appropriate mutants, the second Qβ dipeptide, fMet-Ser, can be correlated with a specific phage gene only indirectly by taking advantage of the fact that Qβ coat protein represses translation of its RNA polymerase cistron (3–5). This translational control operates in several RNA bacteriophages (6, 7) and has been used in other systems to identify the products of *in vitro* translation of the polymerase cistron (3–6, 8–10). In order to make use of this regulatory function in our system, however, the Qβ coat must directly affect translation of the first two codons of the Qβ RNA polymerase cistron. This aspect distinguishes our system from others which viewed initial phage peptides, but derived them from catalytically synthesized phage proteins (4, 10). In the present study, the effect of the repressor can be isolated at the initial region of the cistron since elongation and termination do not influence the extent of synthesis.

Correlating the initial peptides with specific Qβ genes, we can take further advantage of the purified synthetic system to allow a single round of translocation to bring the third codon of each Qβ cistron into the codon recognition site of the ribosome. The third codon can easily be identified using the aminoacyl-tRNA binding technique (11). The nucleotide sequence of the initial region of each cistron can be further elucidated with codon-specific classes of tRNA.

In the present work, we show that Qβ RNA-directed synthesis of the dipeptide fMet-Ser is repressed by Qβ coat protein, that the initial amino acid sequence of *in vitro* synthesized Qβ RNA polymerase should be fMet-Ser-Lys, and that a class of Ser-tRNA which responds only to the codons UCU and UCC is incorporated into this initial peptide. These results allow us to predict that the Qβ RNA polymerase cistron will contain the initial nucleotide sequence $\cdots$AUG·UC_C^U·$AA_G^A\cdots$ and to conclude that coat protein repression affects the availability of the initial region of this cistron for translation.

MATERIALS AND METHODS

Materials

Initiation competent *E. coli* MRE-600 ribosomes (free of T factor but containing G factor activity) (12, 13), purified T (14, 15) and G factors (16), purified (on a benzolated DEAE-cellulose column) (17) and unfractionated aminoacyl-tRNAs (15), and purified specific anti-G factor antibody (18), Qβ RNA (13), and Qβ coat protein (19) were prepared as described. Codon-specific, counter current distribution fractions of Ser-tRNA were generously supplied by Dr. B. P. Doctor.

Assays

Duplicate 0.05-ml reaction mixtures contained 0.05 M Tris-acetate, pH 7.2; 0.05 M NH_4Cl; 5.5 mM magnesium acetate; 2.4 *A* 260 units of ribosomes; 2.5 *A* 260 units of Qβ RNA; 15 μg of T factor; 1 mM GTP; 20 pmol of isotopically labeled aminoacyl-tRNA, where indicated; 0.05 *A* 260 units of unlabeled, purified aminoacyl-tRNA, where indicated; and, where indicated, 40 μg anti-G factor gamma globulin or 2 mM fusidic acid. Binding of labeled aminoacyl-tRNA to ribosomes was assayed by the nitrocellulose filter assay of Nirenberg and Leder (11). Synthesis of fMet-[^{3}H]Ser was assayed after alkaline hydrolysis of the peptidyl-tRNAs and acidification of reaction mixtures with small, acidified Dowex-50 columns according to Roufa and Leder (13).

Electrophoretic analysis of peptide products

Radioactive product samples, to which unlabeled standard peptides had been added, were applied approximately 15 cm from the end of a 115-cm Whatman 3 MM paper strip and electrophoresed in 1 M pyridine acetate, pH 3.5, at 3 kV for 15.5 hr. Standard peptide spots were developed with ninhydrin, and 3-cm lanes were cut into 1-cm strips for scintillation counting. fMet-Ala-Lys and fMet-Ser-Lys were synthesized with the guidance of Dr. Irwin Chaiken by coupling fMet-Ala and fMet-Ser with ε-trifluoroacetyl-lysine (Fox Chemical Co.) in an *N,N'*-dicyclohexylcarbodiimide–*N*-hydroxysuccinimide

TABLE 1. *Effect of Qβ coat protein on the Qβ RNA-dependent synthesis of fMet-Ala and fMet-Ser*

Additions	fMet-[^{3}H]Ala Synthesized (Δ pmol)	fMet-[^{3}H]Ser Synthesized (Δ pmol)
None	1.42	1.67
Qβ Coat protein	1.47	0.76

Reaction mixtures contained components and were incubated as indicated under *Methods*. Purified tRNA preparations were used together with fusidic acid, and peptides were assayed using the Dowex-50 procedure. Coat protein preparations were preincubated with Qβ RNA in 0.1 M Tris·HCl, pH 7.2, at a molar ratio of 28 mol coat protein per mol of Qβ RNA at 37°C for 20 min. Background values, obtained in the absence of Qβ RNA, of 0.34 and 0.28 pmol for fMet-Ala and fMet-Ser, respectively, have been subtracted.

reaction. The appropriately unblocked products were separated by electrophoreses at pH 3.5 as noted above, and characterized by amino acid analysis, dilute acid hydrolysis, and carboxypeptidase B digestion.

RESULTS AND DISCUSSION

Correlation of fMet-Ser with the Qβ RNA polymerase cistron by phage coat protein repression

The experiment shown in Table 1 illustrates the effect of phage Qβ coat protein on Qβ RNA-directed synthesis of fMet-Ala and fMet-Ser. Several points can be made. Synthesis of the Qβ coat dipeptide, fMet-Ala, is not affected by the addition of coat protein, whereas synthesis of Qβ-directed fMet-Ser is inhibited >50% by its addition. Since the RNA phage coat proteins do not affect synthesis of coat or maturation proteins (4), this result suggests that fMet-Ser is the N-terminal peptide of Qβ RNA polymerase. The result also indicates that the coat protein repressor affects one of the first steps in the translation of this cistron, influencing the availability of the initial region of this cistron rather than some later step in the elongation or termination of the peptide chain. It is important to note that translational repression is not complete (see Tables 3 and 5) as measured in this system; it varies between 45 and 75% of nonrepressed values. This reflects the fact that these ribosomes are involved in the stoichiometric synthesis of fMet-Ser, and that these effects cannot be amplified by the catalytic incorporation of amino acids as would occur if elongation and termination were permitted.

Translocation dependent binding of Lys-tRNA in response to two Qβ cistrons

We have previously shown that only two dipeptides, fMet-Ala and fMet-Ser, are synthesized in response to Qβ RNA prior to the first round of translocation (1). The sequence studies of Konigsberg, Maita, Katze, and Weber (2), and Hindley and Staples (20) lead us to suggest that the fMet-Ala peptide we observe corresponds to the Qβ coat protein, which begins with the sequence fMet-Ala-Lys-Leu-Glu... According to a two-tRNA translocation model of ribosomal function (1), appearance of the third Qβ coat codon (specifying lysine) at the recognition site on the ribosome requires a one-codon translocation of coat message. By isolating post-translocation ribosomal intermediates formed with purified fMet-, Ala- and Ser-tRNAs, and testing their ability to bind each of the labeled aminoacyl-tRNAs in turn, we should be able to confirm the appearance of a lysine codon on the ribosomal recognition site. A single round of translocation should be accompanied by a shift of the third codon of the Qβ RNA polymerase cistron into a ribosomal recognition site as well. We should then simultaneously be able to identify the third codon directed by the Qβ RNA polymerase cistron.

TABLE 2. *Translocation requirement for Qβ RNA-directed ribosomal binding of Lys-tRNA*

Additions	[^{14}C]Lys-tRNA bound to ribosomes(Δ pmol)
Expt. 1: fMet- and Ser-tRNAs	
G factor	1.89
Anti-G factor	−0.55
Expt. 2: fMet- and Ala-tRNAs	
G factor	1.52
Anti-G factor	−0.51

Reaction mixtures contained components, including purified fMet, Ala- and Ser-tRNAs where indicated, described under *Methods*. After a 15-min preincubation at 37°C in the presence of these unlabeled tRNAs and anti-G or G factor, as indicated, anti-G factor was added to all reaction mixtures to prevent translocation during the subsequent binding reaction. Then [^{14}C]Lys-tRNA was added to each reaction mixture, the incubation continued for 15 min at 37°C, and bound [^{14}C]Lys-tRNA was determined. Background values, always high in the case of lysine (11), of 6.43–7.55 pmol of [^{14}C]Lys-tRNA bound in the absence of Qβ RNA have been subtracted.

Preliminary experiments were performed by testing the ability of Qβ RNA-directed ribosomal intermediates, permitted one round of translocation, to bind each of 19 aminoacyl-tRNAs (cysteine not tested). Extension of the polypeptide chain was prevented by using purified fMet-, Ala-, and Ser-tRNAs to form the initial post-translocation complex. Then anti-G factor antibody was added to reactions to prevent further translocation of the message during the binding tests. Results of these preliminary experiments indicated that only Lys-tRNA was bound to the post-translocation intermediate. That lysine is the third amino acid of both Qβ peptides is

TABLE 3. *Effect of Qβ coat protein on the Qβ RNA-dependent synthesis of fMet-Ser-Lys*

Addition	fMet-[^{3}H]Ser-Lys synthesized (Δ pmol)
None	0.84
Qβ Coat protein	0.45

Reaction mixtures were identical to those described in the legend of Fig. 1 except that Qβ RNA, preincubated with Qβ coat protein as indicated in the legend of Table 1, was employed where noted. Reaction mixtures were analyzed by electrophoresis as described in the legend to Fig. 1 and values corresponding to the tripeptide were calculated from the total radioactivity migrating as fMet-Ser-Lys. Background values, obtained in the absence of Qβ RNA, were negligible and have not been subtracted.

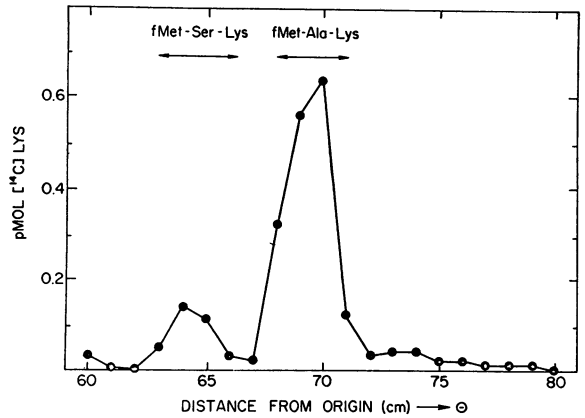

FIG. 1. Electrophoretic analysis at pH 3.5 of Qβ RNA-directed [^{14}C]lysine-containing peptides obtained after a single round of translocation. Products of reaction mixtures scaled up 3-fold, containing components and incubated under the conditions indicated in *Methods* and in the legend to Table 2, were hydrolyzed in 0.1 M NH_4OH at 37°C for 30 min and lyophilized. Positions of standards are indicated by the arrows. No counts were observed in this region in parallel analyses performed in the absence of Qβ RNA.

shown in the experiments illustrated in Table 2 and Fig. 1. In separate experiments, purified fMet- and Ser-tRNAs or fMet- and Ala-tRNAs were supplied, permitting the synthesis of only a single dipeptide product. In both cases Lys-tRNA is bound to the ribosome *only* when a single round of translocation is permitted (Table 2).

The products into which lysine is incorporated have been characterized by a comparison of their electrophoretic mobilities after electrophoresis at pH 3.5 in the presence of standards. As shown in Fig. 1, the lysine-containing products co-electrophorese with fMet-Ala-Lys and fMet-Ser-Lys. Separate experiments with labeled methionine, alanine, and serine also yield products which migrate with the expected standards. As shown in Table 3, analysis of coat protein-repressed reaction mixtures showed reduction in fMet-Ser-Lys synthesis as compared to nonrepressed reactions, indicating that this tripeptide initiates the Qβ polymerase protein.

Coding properties of the initial portion of the Qβ RNA polymerase cistron

The second codon of the Qβ RNA polymerase cistron specifies serine. This amino acid has six codons, comprising three degeneracy classes: UCU, UCC; UCA, UCG; AGU, AGC (21, 22). Using codon-specific fractions of Ser-tRNA, we should be able to identify the specific degeneracy class of serine codons employed in this position. In the experiment shown in Table 4, a counter-current fraction of Ser-tRNA which responds only

Qβ (predicted)	····AUG·UC$^{U}_{C}$·AA$^{A}_{G}$····
PEPTIDE SEQUENCE	···fMET·SER·LYS····
R17 (Steitz)	····AUG·UCG·AAG····

FIG. 2. Comparison of the predicted initial sequence of the Qβ RNA polymerase cistron and that of the analogous R17 region (23).

TABLE 4. *Qβ RNA-directed ribosomal binding of codon-specific serine-tRNAs*

Fraction added	[^{3}H]Ser-tRNA bound to ribosomes (Δ pmol)
Ser-tRNA$_{UCU,C}$	2.66
Ser-tRNA (purified, unfractionated)	1.56

Purified fMet-tRNA was used. Background values, obtained in the absence of Qβ RNA, of 1.11 and 1.56 pmol of Ser-tRNA$_{UCU,C}$ and Ser-tRNA, respectively, have been subtracted.

to the codons UCU and UCC (12) [corresponding to serine counter-current distribution peak II of Söll *et al.* (22)] is bound to the initial Qβ RNA-directed ribosomal complex. As shown, unfractionated Ser-tRNA purified according to Gillam *et al.* (17) (which responds to codons from all three degeneracy classes) is less efficiently bound in response to Qβ RNA. Since Ser-tRNA$_{UCU,C}$ does not respond to the codons UCA, UCG, AGU, or AGC (12), the serine codon in the second position of the Qβ RNA polymerase cistron must be either UCU or UCC. Separate experiments show that incorporation of serine from Ser-tRNA$_{UCU,C}$ into fMet-Ser is repressed by Qβ coat protein (Table 5) and, therefore, indicate that a codon of this degeneracy class is present in the second position of the RNA polymerase cistron. Knowing this, and taking advantage of the fact that there is only one codon for methionine and only two for lysine, we may predict a rather narrow range of base sequences corresponding to the initial region of the Qβ RNA polymerase cistron.

The predicted sequence ···AUG·UC$_C^U$·AA$_G^A$··· is shown in Fig. 2 and compared with the nucleotide sequence determined for the initial region of the bacteriophage R17 RNA polymerase cistron (23). The first three amino acids of Qβ RNA polymerase are apparently identical to those of the R17 RNA polymerase; but a different degeneracy class of serine codon is employed in the second position of each initiation region. Although both of these RNA polymerases perform very similar functions after phage infection, each is quite specific in that only homologous species of RNA are replicated (24). Further, the translation of both is repressed by the protein subunits of their respective phage coats (3–10). We have seen above that one of the first steps in the translation process

TABLE 5. *Effect of Qβ coat protein on the Qβ RNA-dependent incorporation of serine from Ser-tRNA$_{UCU,C}$ into fMet-Ser*

Addition	fMet-[^{3}H]Ser synthesized (Δ pmol)
None	0.84
Qβ Coat protein	0.24

Reaction mixtures contained components and were assayed according to procedures indicated in the legend to Table 1. Purified fMet-tRNA was used together with codon-specific Ser-tRNA$_{UCU,C}$. Background values of 0.42 pmol for reactions were obtained in the absence of Qβ RNA and have been subtracted.

must be affected. Inasmuch as the site of interaction of the coat protein repressor and RNA message *may* be near or at the RNA polymerase initiation region, its sequence is of considerable interest. It will be particularly useful to compare the sequence of this region, especially that portion to the left of the AUG codon—the intercistronic gap—with that of the R17 message. The ability of the coat proteins of these two phages to form heterologous phage-like particles with one another's RNA (25), despite differences in amino acid composition and sequence (2, 26), suggests there must be certain similar regions in both coat proteins and RNAs. Similarities in the coat proteins have been pointed out by Konigsberg *et al.* (2). In this connection, the basic similarity of the predicted and established initial sequence of both RNA polymerase cistrons, despite the apparent species specificity of coat protein repression (3, 5), is noteworthy. It is possible that this region is the site of interaction of the coat repressor. The region to the left of the AUG in this cistron may be involved in conferring specificity on this interaction. This region does not contain the otherwise ubiquitous UUUGA sequence (23), a sequence present to the left of the other R17 cistrons (23). These other cistrons are not subject to repression by coat protein, and it is possible that the sequence substituted for UUUGA near the RNA polymerase cistron confers specificity on its interaction with repressor.

Finally, we may consider the maturation-protein cistron. Our cell-free system yields only two dipeptides, which correspond to a minimum number of cistrons. It is possible that the Qβ maturation protein is also initiated by either of these amino acid sequences, but more likely its cistron is simply unavailable for translation under the conditions tested. Recognition of the F2 maturation cistron, for example, required rather special conditions altering the state of the message or the source of the ribosomes employed (3, 4, 23). The initial sequence of this protein is particularly important, however, for it can ultimately be correlated with nucleotide sequence studies, which are progressing from the 5′-end of Qβ RNA (27, 28), and will thereby define the limit of the untranslated or silent region of this message.

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