Studies of Temperature Sensitive Mutants of Bacteriophage Qβ, Defective in both Replication and Translation

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SUMMARY

Temperature sensitive mutants of bacteriophage Qβ have been isolated which fail in the synthesis of their virus RNA at the non-permissive temperature (42 °C). Nine mutants have been studied in some detail. Cells infected with these mutants at 37 °C and incubated long enough to produce substantial amounts of Qβ RNA cease Qβ RNA replication when shifted to 42 °C. The mutants can be classified into 3 groups according to the amount of Qβ RNA replicase activity exhibited in extracts from infected cells isolated at various times after shift to 42 °C: in group 1 mutants, enzyme activity is the same, regardless of the time of isolation after shift; in group 2 mutants enzyme activity increases with time of isolation after shift; in group 3 mutants, enzyme activity decreases with time of isolation after shift. Synthesis of all virus proteins is suppressed at 42 °C in cells infected with group 1 or group 3 mutants. In cells infected with group 2 mutants, synthesis of Qβ RNA replicase subunit β is increased, but synthesis of other virus proteins is depressed at 42 °C. The inhibition of virus RNA and protein synthesis is reversible. A detailed analysis of these experiments suggests that a defective Qβ RNA replicase is involved in the inhibition of both virus RNA and protein synthesis.

INTRODUCTION

The nucleic acid of RNA bacteriophages serves both as a messenger for protein synthesis and as a template for its own replication. Translation proceeds from the 5’ to the 3’ end of the RNA (Thach et al. 1965), but replication proceeds in the opposite direction (August et al. 1968). How these processes are interrelated is not understood in detail.

While searching for temperature-sensitive mutants of the bacteriophage Qβ we isolated quite a number that are defective in their ability to induce virus RNA replication. Surprisingly, in all these replication-defective mutants, virus protein synthesis is affected as well as RNA synthesis and both defects are reversible. Such mutants should be useful in studying replication and translation interactions. The purpose of this paper is to report characteristics of these mutants. Studies of replication-defective temperature sensitive mutants of the phages f2 (Horiuchi, Lodish & Zinder, 1966) and MS2 (Pfeifer, Davis & Sinsheimer, 1964),

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but not of phage Qβ, have been reported. Studies of Qβ mutants may have the advantage that information about Qβ replicase is especially complete and definitive.

We are unable to explain the molecular mechanism responsible for the simultaneous inhibition of replication and translation. However our data are consistent with the proposals of Kolakofsky & Weissmann (1971) for clearing of ribosomes from Qβ RNA prior to the onset of Qβ RNA replication.

**METHODS**

**Chemicals.** All the chemicals used were analytical grade. Amino acids were purchased from Mann Research Laboratories, New York. ATP (disodium salt), GTP (trisodium salt), 2-phosphoenolpyruvic acid (trisodium salt) and pyruvate kinase were from Calbiochem, Los Angeles. Egg white lysozyme, pancreatic RNase A, and DNase I were from Worthington, Freehold, New Jersey. [3H]-uracil, [14C]-uracil, [3H]-lysine and rifampin were obtained from Schwarz Bioresearch, Orangeburg, New York. Gliotoxin was a gift from Dr J. K. Smith, Jun.

**Virus and bacteria.** Phage Qβ was from a stock in use in our laboratory for a number of years. To prepare new phage stock, cultures of *Escherichia coli* A19 or Q13 were infected at an E50 = 0.5 with wild type or mutant phage Qβ at multiplicity of infection (m.o.i.) of 10. At 3 h post-infection, 1 ml of 10 mg/ml lysozyme and 1 ml of 0.25 m-disodium-EDTA, pH 7.8, were added per 100 ml culture. After incubating for 5 min at room temperature, 1 ml of chloroform was added, the mixture was chilled in ice and was centrifuged to remove debris. The supernatant fluid was the stock phage solution. Phage titres were determined by the standard agar overlay technique.

Mutants were obtained by treatment of purified Qβ virus with 2 m-KNO₃ in 0.25 m-acetate buffer, pH 4.6, for 100 min at 23 °C as described by Tessman (1959) and Horiuchi & Matsuhashi (1970). The survivors: 5 x 10⁶ p.f.u./ml of 10¹² p.f.u./ml starting virus, were plated on *Escherichia coli* M27 at 37 °C. Each plaque was stabbed with a sterile toothpick which was then dipped into 0.5 ml of tryptone broth. A small loop (< 10 µl) of the sample-containing broth was spotted on a bacterial lawn and incubated at 34 °C and 42 °C. If no virus growth occurred at 42 °C, a small portion of the spot which formed at 34 °C was used to infect 5 ml of a culture of M27 bacteria at 5 x 10⁷ cells/ml. After 3 to 5 h at 34 °C with aeration, EDTA to 0.002 M and chloroform to 1% were added, and cell debris was removed by centrifuging.

**Assay conditions.** Radioactive uracil and lysine incorporation were measured according to the procedures described by Passent & Kaesberg (1971) with the exception that TPG medium (Vinuela, Algranati & Ochoa, 1967) was used instead of tryptone broth.

Temperatures of incubation, time of addition of chemicals, and radioactive materials are given in descriptions of individual experiments.

To analyse the radioactive polypeptides synthesized upon virus infection, infected cells were chilled in ice after the incorporation period and were harvested by centrifuging and were solubilized as described by Jockusch, Ball & Kaesberg (1970). Samples of the solubilized cells were subjected to electrophoresis on 10% polyacrylamide gels containing SDS, as described by Strauss & Kaesberg (1970), and fractions were obtained with an automatic gel crusher. One fraction corresponded to a gel length of approx. 1 mm and contained 0.33 ml of water delivered automatically by the gel crusher. The radioactive material was eluted from the gel fractions during incubation for 3 h at 60 °C. After cooling, 10 ml of scintillation fluid (consisting of 50% toluene and 50% ethoxyethanol plus the appropriate scintillators) was added to each fraction and these were counted in a liquid scintillation counter.
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*Viral RNA synthesis in infected cells was measured by the incorporation of [14C]-uracil. Rifampin was used to suppress *Escherichia coli* DNA-dependent RNA synthesis without having any direct effect on virus RNA synthesis (Hartman et al. 1967). Separate cultures of *E. coli* A19 cells were infected with wild type Qβ and the 9 mutants under investigation and were grown at 42°C. Twelve min after infection, rifampin was added to a concentration of 80 μg/ml. Four minutes later, [14C]-uracil was added to a final concentration of 0.25 μCi/ml and at 90 min after infection TCA precipitable radioactivity was measured as described in Methods.*

**Qβ RNA replicase was assayed in crude extracts prepared as follows.** Samples of infected cells were withdrawn at appropriate times and centrifuged at 12,000 rev/min in a Sorvall SS-34 centrifuge rotor at 4 °C for 15 min. The pellet from 5 ml of culture was resuspended in 0.3 ml TG buffer (containing 0.01 M-tris, 0.001 M-EDTA, 0.01 M-mercaptoethanol, and 25% glycerol, pH 7.5). To 0.3 ml of cell suspension were added 0.01 ml of 0.25 M-EDTA and 0.01 ml of 10 mg/ml lysozyme. The mixture was frozen and thawed three times. The resulting suspension was then incubated for 10 min at room temperature. Then to it, 0.01 ml of 0.1 M-MgCl₂ and 0.01 ml of 1 mg/ml DNase I were added and incubated at 4 °C for 10 min. An assay mixture was prepared to contain in 0.2 ml: 10 μg of Qβ RNA, 160 nmol each of ATP, GTP, and CTP, 10 nmol of [3H]-UTP (sp. act. 0.48 mCi/μmol), 1 μg rifampin, 0.005 M-phosphoenolpyruvate, 10 μg pyruvate kinase, 10 μg DNase I, 0.01 M-MgCl₂, 0.025 M-Mg-mercaptoethanol, 0.125 M-tris, pH 7.5, and a 30 μl sample of crude extract. After incubation at 37 °C for 10 min the reaction was stopped by the addition of 3 ml of 5% TCA containing 0.02 M-sodium pyrophosphate and 0.1 ml of 1% bovine serum albumin. The mixture was cooled and the precipitate was collected on glass filter paper, washed twice with TCA, dried, and counted in a liquid scintillation counter. Alternatively, Qβ RNA replicase was purified as described by August et al. (1968) and it was assayed as described above with the exception that there was no pyruvate kinase, phosphoenolpyruvate or DNase I in the incubation mixture.

### Results

All the temperature sensitive mutants of bacteriophage Qβ pertinent to the present work were isolated after HNO₂ mutagenesis of wild type phage as described in Methods. These mutants do not grow, or grow poorly, at 42 °C, but grow readily at 37 °C. In the following sections we describe properties of 9 of these mutants, selected for detailed study. These are mutants C1, C2, C3, C4, C5, C6, C7, C8 and C9. Genetic experiments, to be described elsewhere, indicate that all these mutants have a defect in their replicase cistron.

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### Table 1. Virus RNA synthesis and Qβ replicase activity in wild type and mutant phage infected cells at 37°C and 42°C*

<table>
<thead>
<tr>
<th>Phage</th>
<th>Uracil incorporation (ct/min) 37°C cells</th>
<th>UTP incorporation (ct/min) 37°C cells</th>
<th>Uracil incorporation (ct/min) 42°C cells</th>
<th>UTP incorporation (ct/min) 42°C cells</th>
</tr>
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<tr>
<td>Wild type uninfected</td>
<td>8054</td>
<td>6415</td>
<td>62028</td>
<td>56444</td>
</tr>
<tr>
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<td>8548</td>
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<td>7495</td>
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* Viral RNA synthesis in infected cells was measured by the incorporation of [14C]-uracil. Rifampin was used to suppress *Escherichia coli* DNA-dependent RNA synthesis without having any direct effect on virus RNA synthesis (Hartman et al. 1967). Separate cultures of *E. coli* A19 cells were infected with wild type Qβ and the 9 mutants under investigation and were grown at 42 °C. Twelve min after infection, rifampin was added to a concentration of 80 μg/ml. Four minutes later, [14C]-uracil was added to a final concentration of 0.25 μCi/ml and at 90 min after infection TCA precipitable radioactivity was measured as described in Methods.
Fig. 1. Virus RNA synthesis at 37 °C or after shift-up from 37 °C to 42 °C. Cells were infected at 37 °C with mutant or wild type virus. Rifampin (final concentration of 80 μg/ml) was added at 25 min. At 35 min, one portion of an infected culture was shifted to 42 °C, while another portion was kept at 37 °C. At 40 min, [3H]-uracil was added to each portion to give a concentration of 5 μCi/ml. Incorporation of [3H]-uracil was assayed as described in Methods. In one experiment, chloramphenicol was added at 38 min. (a) [3H]-uracil incorporation at 37 °C. (b) [3H]-uracil incorporation at 42 °C. □—□, wild type; ○—○, C2; △—△, C3; ×—×, C3 + chloramphenicol.

Synthesis of virus RNA and virus replicase at non-permissive temperature

The defining characteristic of these mutants is failure to synthesize Qβ RNA at 42 °C. Table I shows that cultures infected with any of the 9 mutants incorporate [14C]-uracil very poorly at 42 °C, although wild type infected cells incorporate [14C]-uracil readily.

A closely related characteristic is the absence of replicase activity in infected cells grown at 42 °C. The Table shows that all 9 mutants induce very little replicase when infected cells are grown at 42 °C throughout the experiment.

The results shown in Table 1 are, of course, interdependent. In the absence of Qβ RNA synthesis, very little replicase will exist in infected cells (because Qβ RNA is the messenger RNA for subunit β of replicase). Conversely, in the absence of replicase, no Qβ RNA replication will occur.

Temperature shift-up experiments

To test whether RNA synthesis occurs at 42 °C in cells known to contain replicase, cells infected and grown at 37 °C were shifted at a later time to 42 °C, and were then analysed for uracil incorporation as before. It is known that in cells infected with wild type Qβ, replicase synthesis can be detected within 15 min after infection; it reaches a maximum at about 35 min, and is repressed thereafter (August et al. 1968; Horiuchi & Matsushashi, 1970). Qβ RNA synthesis occurs during the entire period between the onset of replicase synthesis and cell lysis.

Fig. 1 (a) shows that at 37 °C, cells infected with mutants C2, C3, or wild type Qβ continue to synthesize RNA throughout the entire growth period after infection. However, a shift to 42 °C at 35 min after infection causes inhibition of virus RNA synthesis in cells infected
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Fig. 2. Kinetics of $Q\beta$ RNA replicase synthesis in infected cells after shift-up to 42 °C. *Escherichia coli* cells were infected with each mutant separately at 37 °C. At 15 to 17 min after infection, cells were shifted to 42 °C. Crude extracts were prepared from samples of infected cells and $Q\beta$ RNA replicase was assayed by measuring [H]-UTP incorporation in the presence of rifampin at 37 °C as described in Methods. In each set of kinetic experiments, the first point represents $Q\beta$ RNA replicase activity just before shift-up. (a) Group 1: $\bullet$, C4; $\circ$, C1. (b) Group 2: $\bullet$, C7; $\circ$, C8. (c) Group 3: $\circ$, C9.

Results of this experiment for 6 of the mutants are shown in Fig. 2. It may be seen that these mutants can be classified into 3 groups according to the kinetic pattern they exhibit. Group 1: replicase activity does not change with time after shift-up to 42 °C. Mutants C1, C2, and C3 are included in this group. Cells infected with mutants C4, C5, and C6 (not shown in Fig. 2) exhibit a similar kinetic pattern. The kinetic pattern is the same when chloramphenicol is

with mutants C2 and C3 while wild type $Q\beta$-infected cells continue to synthesize virus RNA after shift-up (Fig. 1b). Similar results were obtained when the other mutants were used. Similar inhibition of RNA synthesis at 42 °C was obtained with shift-up at 22 min after infection. Addition of chloramphenicol to mutant C3-infected cells at 38 min after infection does not cause inhibition of RNA synthesis at 37 °C (Fig. 1a). RNA synthesis also proceeds (although more slowly) when chloramphenicol is added to either C3-infected or wild type $Q\beta$-infected cells at 22 min after infection (data not shown). We conclude that in mutant-infected cells a shift to 42 °C, either late or early, causes inhibition of virus RNA synthesis. This inhibition is not merely a consequence of an inhibition of replicase synthesis itself, because at 37 °C, RNA synthesis persists in the presence of chloramphenicol, a potent inhibitor of protein synthesis.

To test whether replicase is synthesized and survives at 42 °C in cells infected with the mutants, enzyme activity was measured in crude extracts prepared from samples of infected cells, as described in Methods. The time course of replicase activity of the 9 mutants was measured after shifting up to 42 °C at 18 min after infection, at which time $Q\beta$-infected cells already contain one-third of their maximum amount of replicase (Horiuchi et al. 1971). Results of this experiment for 6 of the mutants are shown in Fig. 2. It may be seen that these mutants can be classified into 3 groups according to the kinetic pattern they exhibit. Group 1: replicase activity does not change with time after shift-up to 42 °C. Mutants C1, C2, and C3 are included in this group. Cells infected with mutants C4, C5, and C6 (not shown in Fig. 2) exhibit a similar kinetic pattern. The kinetic pattern is the same when chloramphenicol is
Fig. 3. Virus protein synthesis at 37 °C or after shift-up from 37 °C to 42 °C. Escherichia coli cells were infected at 37 °C with mutant or wild type virus. Rifampin (final concentration of 100 μg/ml) was added at 10 min after infection. At 18 min, one portion of cells was shifted to 42 °C, while another portion was kept at 37 °C. At 21 min, [3H]-lysine was added to each portion to give a concentration of 12.5 μCi/ml. Incorporation of [3H]-lysine was determined by measuring hot TCA precipitable radioactivity as described in Methods. (a) [3H]-lysine incorporation at 37 °C. (b) [3H]-lysine incorporation at 42 °C. △—△, wild type; □—□, C3; ○—○, C7; ●—●, C9.

We conclude that group 1 mutants do not synthesize replicase at 42 °C regardless of the presence of Qβ RNA. Since the kinetic pattern of enzyme activity after shift-up does not change in the presence of chloramphenicol it is ruled out that in these mutants at 42 °C replicase is synthesized at the same rate that it becomes inactivated. Thus, at 42 °C group 1 mutants are defective in synthesis of replicase as well as being unable to use replicase already synthesized. Group 2 mutants are not defective in the production of replicase at 42 °C when Qβ RNA is present as a messenger. However, the replicase is unable to function in cells at 42 °C. The replicase produced at 37 °C in cells infected with the group 3 mutant is thermostable and becomes inactivated in cells at 42 °C. Like the group 1 and group 2 mutants the replicase is unable to function in cells at 42 °C.

In vitro Qβ RNA synthesis

We have tried a variety of experiments designed to detect differences in the in vitro characteristics of wild type and mutant replicases. Except that the replicase of the group 3 mutant is heat labile, we have been unable to find in vitro characteristics of any of the mutant replicases that distinguish them from the wild type enzyme.
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In vivo synthesis of virus proteins

In our shift-up experiments we concluded that the synthesis of replicase is inhibited at 42 °C in cells infected with group 1 or group 3 mutants. Can we demonstrate inhibition of synthesis of replicase subunit β and also that of other virus proteins at 42 °C? To answer this question phage-directed protein synthesis was measured by assay of [3H]-lysine incorporation in infected cells after shift-up at a time when there was considerable Qβ RNA present as a messenger. Cells infected with the mutants and wild type incorporate [3H]-lysine at 37 °C (Fig. 3a). Fig. 3b shows that cells infected with mutants C3 (group 1) and C9 (group 3) do not incorporate [3H]-lysine at 42 °C, while mutant C7 (group 2) incorporates some [3H]-lysine, albeit at a low level. On the other hand, wild type infected cells incorporate [3H]-lysine readily at 42 °C. Like C3, cells infected with mutants C1, C2, C4, C5 and C6 (group 1) also do not incorporate [3H]-lysine at 42 °C. Thus, in group 1 and group 3 mutants (and to some extent in group 2 mutants) protein synthesis as well as RNA synthesis is inhibited at the non-permissive temperature.

We have already seen in the experiments involving chloramphenicol that, at 37 °C, Qβ RNA synthesis can continue even though protein synthesis has been inhibited. Thus cessation of protein synthesis does not, necessarily, result in inhibition of Qβ RNA synthesis, even though these events are coupled at 42 °C with our mutants. Conversely, can Qβ RNA synthesis be inhibited without the concomitant inhibition of viral protein synthesis? We examined this possibility by blocking RNA synthesis at 37 °C with the drug gliotoxin and then measuring virus protein synthesis. Gliotoxin has been shown to inhibit poliovirus RNA replication without having any effect on poliovirus protein synthesis (Miller, Milstrey & Trown, 1968). With our experimental conditions, gliotoxin at 100 μg/ml inhibits Qβ RNA synthesis very effectively at 37 °C. Control experiments involving in vitro protein synthesis showed that gliotoxin has no effect on Qβ RNA-directed protein synthesis. We find that cells infected with mutants C3, C7, C9 and wild type continue to incorporate [3H]-lysine at 37 °C at their usual level in the presence of gliotoxin. It may be concluded that, in cells infected with these mutants, inhibition of virus RNA synthesis does not obligatorily cause inhibition of virus protein synthesis. The inhibition of [3H]-lysine incorporation at 42 °C must be due to some specific defect in virus protein synthesis.

Since coat protein constitutes 80 to 85% of the total virus protein synthesized during Qβ phage infection, measurement of lysine incorporation provides information mostly about its synthesis. In order to measure the synthesis of replicase subunit β, maturation protein and polypeptide IIb we analysed extracts of infected cells by polyacrylamide gel electrophoresis. Fig. 4 shows the polyacrylamide gel patterns of virus proteins synthesized by mutants C3 (group 1), C7 (group 2), and C9 (group 3) at 37 °C and after upshift to 42 °C. At 37 °C the patterns are indistinguishable from those of wild type Qβ. In accordance with the characterization of Jockusch et al. (1970) and Horiuchi, Webster & Matsuhashi (1971), the 4 radioactive peaks, indicated by the arrows, represent replicase polypeptide β, maturation protein, polypeptide IIb and coat protein in order of increasing mobility. At 42 °C, in cells infected with mutant C3 (group 1) and mutant C9 (group 3) the amount of each virus protein synthesized is much lower than that synthesized at 37 °C. Thus, synthesis of all the proteins coded by mutant C3 (group 1) and C9 (group 3) RNA are suppressed at 42 °C. This is in agreement with the data obtained from the kinetics of [3H]-lysine incorporation for all the group 1 mutants. However, in mutant C7 the amount of replicase peptide β synthesized at 42 °C is even higher than that synthesized at 37 °C, whereas coat protein synthesized at 42 °C is much lower in amount than that obtained at 37 °C.
Fig. 4. Polyacrylamide gel electrophoresis patterns of (a) mutant C3, (b) mutant C7 and (c) mutant C9 proteins, synthesized in vivo from 20 min to 40 min after infection either at 42 °C or in the presence of gliotoxin at 37 °C. Cells were infected at 37 °C with mutant virus. Rifampin was added at 10 min after infection to give a concentration of 100 µg/ml. At 20 min after infection, one portion of the culture was shifted to 42 °C while another portion was kept at 37 °C and gliotoxin was added to give a concentration of 100 µg/ml. At 23 min [3H]-lysine was added to both portions to a concentration of 12.5 µCi/ml. At 40 min both portions were treated as described in Methods and were subjected to electrophoresis on 8 cm, 10 % polyacrylamide-SDS gels. Electrophoresis was at 3 mA/gel and 18 V until the blue dye marker reached the end of the gel. The arrows in panel (a) indicate the identification of the peaks. ———, virus proteins synthesized at 42 °C; ———, virus proteins synthesized at 37 °C in the presence of gliotoxin.

We conclude that coat protein synthesis in these mutants is very much depressed at 42 °C. Furthermore, in group 1 and group 3 mutants synthesis of replicase polypeptide β at 42 °C is depressed as well. However in group 2 mutants the synthesis of polypeptide β is enhanced at 42 °C in striking agreement with our previous conclusions regarding synthesis of replicase itself.
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Fig. 5. Effect of temperature shift-up and shift-down on [3H]-uracil incorporation in cultures infected (a) with mutant C7 or (b) with mutant C3. Rifampin (to 80 μg/ml) and [3H]-uracil (to 5 μCi/ml) were added to mutant infected cultures at 37 °C at 10 min and 14 min after infection, respectively. The cultures were shifted up to 42 °C at 22 min and down to 37 °C at 38 min. Incorporation of [3H]-uracil was measured as described in Methods. In a separate culture infected with mutant C3, rifampin was added at 10 min after infection at 37 °C and the culture was shifted up to 42 °C at 21 min after infection. At 27 min after infection, chloramphenicol was added to give a final concentration of 70 μg/ml and immediately the culture was shifted down to 37 °C. Three min later, [3H]-uracil (to 5 μCi/ml) was added and the kinetics of incorporation of [3H]-uracil were measured.

Temperature shift-up, shift-down experiments

Thus far, we have shown that in cells infected with these mutants, virus RNA synthesis and virus coat protein synthesis are inhibited at 42 °C. Are these effects reversible? Experiments relating to reversibility are both technically difficult and are difficult to interpret. This is because cellular and virus synthetic processes are closely interrelated and must be sorted out and also because virus nucleic acid synthesis and protein synthesis are interdependent—effecting changes in one affects both, usually in a cumulative way. Nevertheless, we find, unequivocally, that for both virus RNA and virus protein synthesis, inhibition caused by shifting to non-permissible temperature is reversed by shifting back to permissible temperature.

We selected two mutants, C3 (representative of group 1) and C7 (representative of group 2) in order to study reversibility of the inhibition of virus RNA and protein synthesis.

RNA synthesis was determined by measuring [3H]-uracil incorporation in the presence of rifampin after shift-up to 42 °C and subsequent shift-down to 37 °C. Fig. 5 shows that incorporation of [3H]-uracil by both mutant C3 and mutant C7 is stopped soon after shift-up to 42 °C, but is resumed shortly after shift-down to 37 °C. Upon addition of chloramphenicol during shift-down, RNA synthesis is nevertheless resumed. This resumption of RNA synthesis after shift-down in the presence of chloramphenicol suggests that the reversal of inhibition is not due to synthesis of new enzyme. The RNA synthesized after shift-down has the electrophoretic properties of authentic Qβ RNA.

In order to determine the reversibility of the inhibition of protein synthesis, virus protein
Fig. 6. Effect of temperature shift-up and shift-down on the kinetics of [3H]-lysine incorporation in cells infected with mutant C7 or with mutant C3. In (a), rifampin (to 80 μg/ml) and [3H]-lysine (to 12.5 μCi/ml) were added at 8 min and 14 min, respectively, after infection with mutant C7. The culture was shifted up to 42 °C at 23 min and down to 37 °C at 38 min. [3H]-lysine incorporation was measured as described in Methods. In (b), cells at 37 °C were shifted up to 42 °C at 20 min post-infection. One portion of the culture was then shifted down to 37 °C at 35 min after infection, while an equal portion was kept at 42 °C. At 38 min, [3H]-lysine (to 12.5 μCi/ml) was added to each of the cultures. Samples were withdrawn from both infected cultures at the indicated times and incorporation of [3H]-lysine was measured.

Fig. 6 (a) shows [3H]-lysine incorporation by mutant C7 before and after shift-down to 37 °C. Fig. 6 (b) shows [3H]-lysine incorporation by mutant C3 after shift-down. It may be seen that in both mutant C3 and C7, incorporation of [3H]-lysine is suppressed at 42 °C, but is resumed after shifting down to 37 °C. The predominant protein synthesized after shift-down has the electrophoretic properties of virus coat protein.

DISCUSSION

Studies of nine temperature sensitive mutants of bacteriophage Qβ deficient in RNA replication have been described. Surprisingly all 9 mutants are also defective in virus protein synthesis at 42 °C. Our data indicate that the inhibition of virus RNA at 42 °C is due to the presence of inactive RNA replicase. Genetic experiments, to be described elsewhere, in which group 1 mutants complement very well with both maturation protein and coat protein mutants, indicate that at least group 1 mutants have a single defect in their replicase cistron. This is supported by the high reversion frequency of group 1 mutants. As we will discuss below similar defects may occur in groups 2 and 3 mutants. This suggests that temperature sensitive mutations in the replicase cistron frequently result in a particular kind of defect, one that affects not only replication of Qβ RNA but also its translation.

According to the Kolakofsky & Weissmann (1971) hypothesis, active Qβ RNA replicase binds to the coat protein initiation site (as well as to the 3' terminus) of Qβ RNA and this binding blocks further entry of ribosomes into the coat protein initiation site (Kolakofsky & Weissmann, 1971; Weber et al. 1972). As ribosomes, already engaged in translation, are
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cleared from the template, Qβ replicase can proceed along the RNA chain and synthesize the complementary minus strand. We may speculate that with group 1 mutants, when the temperature is brought to 42 °C the Qβ RNA replicase is changed in conformation in such a way that it can no longer catalyse replication even though binding of the enzyme to Qβ RNA (both plus and minus strands) occurs. Thus, virus RNA synthesis is inhibited at 42 °C. Since enzyme molecules remain bound to the RNA plus strand at the coat protein initiation site, entry of ribosomes is blocked and as a result, coat protein synthesis is inhibited (Ball & Kaesberg, 1973). Since the coat protein cistron is not translated, translation of the replicase cistron also does not occur. Thus subunit β of Qβ is not synthesized and active enzyme is not made.

The situation with mutant C7 (group 2) is somewhat different. When the temperature of infected cells is raised to 42 °C RNA synthesis and coat protein synthesis are inhibited but subunit β of Qβ replicase continues to be synthesized and Qβ replicase continues to be made (even though at 42 °C it is inactive in vivo). We may invoke the same hypothesis for inhibition of Qβ RNA and coat protein synthesis as we have for the group 1 mutants but an additional mechanism is required to account for the continuance of synthesis of subunit β. With wild type Qβ (and presumably also with group 1 mutants) translation of coat protein has a polar effect on translation of subunit β; that is, at least a part of the coat cistron must be translated before the subunit β cistron is translatable. It is believed that the process of coat protein translation leads to a refolding of the RNA in such a way that the subunit β cistron becomes accessible to ribosomes. It may be that the mutational events that occurred in the creation of mutant C7 also resulted in an RNA configuration suitable for the translation of subunit β.

Mutant C9 (group 3) differs from the group 1 mutants primarily in that its replicase is thermostable in vivo (as well as in vitro). It is well known that Qβ replicase from the wild type virus is unstable in vitro. However this is believed to be a consequence of the lability of its subunit Tα, one of the elongation factors involved with protein synthesis in Escherichia coli. Presumably wild type Qβ replicase is stable in vivo because there is a plentiful supply of Tα available in the host cells. The instability of the Qβ replicase of mutant C9 in vivo must be due to the lability of its subunit β.

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REFERENCES


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