Control of Replication in RNA Bacteriophages

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The rates of viral RNA and protein syntheses for wild-type RNA bacteriophages and their nonpolar, coat protein amber mutants were determined in amber suppressor (S26R1E, Su-1 and H12R8a, Su-3) and nonsuppressor (AB259, S26, and Q13) strains of *Escherichia coli* in the presence of rifamycin. It was demonstrated that the rates of synthesis of phage-specific replicase and RNA minus strands drop off concurrently in both wild-type and coat protein mutant-infected Su⁻ and Su⁺ cells after 10 and 15 min postinfection, respectively. The rate of synthesis of RNA plus strands started to decline 5 to 10 min later in both cases. Excessive synthesis of replicase in the coat protein mutant-infected cells was accompanied by a similar overproduction of RNA minus strands, but not of plus strands. Partial suppression of protein synthesis in wild-type phage-infected cells abolishing coat protein control over replicase accumulation led to prolongation of replicase synthesis. Such an effect was observed also in coat protein mutant-infected cells, indicating that the excess of replicase itself may be capable of suppression of replicase synthesis in the absence of coat protein. The prolongation of replicase synthesis was followed by the prolonged synthesis of RNA minus strands in both cases. Moreover, replicase and minus strands were formed in nearly equal amounts when protein synthesis was partially inhibited. Assuming functional instability of phage RNAs, the observed coupling of replicase and minus-strand RNA synthesis offers a possibility for control of viral RNA replication by means of control of replicase synthesis on the translational level. A hypothesis is put forward to explain the molecular mechanism of such coupling between the syntheses of replicase and RNA minus strands.

Replication of RNA bacteriophages comprises a series of interrelated processes and apparently provides an example of a very primitive, living self-regulating system. In spite of the large body of literature existing on the subject (for references see 10, 32), no general picture of regulation has been elaborated so far. In contrast to the control of protein synthesis on phage templates, regulation of RNA synthesis has until now received comparatively little attention, except for the demonstration of the predominant synthesis of RNA plus strands over minus strands (2).

Expression of viral genes is controlled by phage-specific proteins which are bound to the RNA template to form translational repressor complexes. Repressor complex I formed by the coat protein is unable to initiate the synthesis of the phage-coded subunit of replicase (further designated as replicase; 4, 25, 26). Repressor complex II formed by the replicase presumably abolishes initiation of both coat protein and replicase synthesis (12, 13).

In this paper evidence is presented demonstrating that production of viral RNA is regulated at the translational level. Such control may be possible if replicase and RNA syntheses are

somehow coupled. According to our hypothesis, initiation of RNA minus strands requires unfolding of the 3'-terminal part of the RNA plus template, which is probably effected by the ribosome during translation of the replicase cistron. Initiation of the plus-strand synthesis at the same time appears to be independent of the replicase cistron translation. If RNA minus strands are functionally unstable, the rate of plus-strand synthesis must decrease after a period equal to the life time of an RNA minus strand in the absence of replicase synthesis. Regulation of replicase synthesis is sufficient in such a case to exert effective control over viral RNA replication.

The fact that the coat protein controls translation of the replicase cistron is well established (for references, see 10, 32). The role of replicase as a potential inhibitor of its own synthesis is a subject of discussion in the present paper, in the light of our findings on RNA and protein synthesis in cells infected with the nonpolar coat protein amber mutants. In addition, data on the readthrough protein A_n synthesis were used to substantiate our assumption. A_n protein is synthesized in amber suppressors after suppression

of UAG, the terminating codon for A protein in the phages of the first serological group (20).

Experiments on the replication of phage MS2 and its amber mutants were used to illustrate our statements. At the same time, similar data were also gained for other bacteriophages of the first ($\phi 2$, fr, R17) and third (Q β) serological groups.

MATERIALS AND METHODS

Bacteria, bacteriophages, and growth conditions. Phage MS2, originally provided by A. Clark, was from A. S. Krivisky (Moscow). MS2 amber mutants were from the following sources: the A protein mutant *am302*, synthesizing 90% A protein fragment in nonpermissive cells (29), and the nonpolar coat protein mutants *am601* and *am623*, carrying mutations in positions 70 and 50, respectively (21), were kindly provided by W. Fiers (Ghent). The double mutant *f2sus11*, carrying amber mutations in the A protein cistron and the coat protein cistron (position 70; 33), was obtained from the Institute of Microbiology (Riga). Mutants were grown according to the method of Tooze and Weber (28) and contained less than 0.1% revertants. The sources of origin of other phages are indicated elsewhere (1). The following non-suppressor strains of *Escherichia coli* were used: AB259 Hfr 3000 Thi⁻ and Q13 Hfr RNase I⁻ PNPase⁻ Met⁻ Tyr⁻ (1). The nonsuppressor strains S26 and suppressor strains S26R1E Su-1 and H12R8a Su-3, originally isolated by A. Garen, were from W. Fiers. The cells were grown on a rotatory shaker (240 rpm) at 37°C in TPG medium (24) with appropriate supplements for auxotrophs, or in TPG plus tryptone, containing 2 g of tryptone (Difco) per liter. At 2×10^8 to 4×10^8 cells per ml, cultures were infected at a multiplicity of infection of 10. To determine the rate of protein (in TPG medium only) and RNA (in both media) syntheses, the infected cells were treated with rifamycin (Gee Lawson; final concentration, 100 μ g/ml). Chloramphenicol (Calbiochem), streptomycin, and tetracycline (medical grade) were used as protein synthesis inhibitors. To determine the efficiency of protein synthesis inhibition at different drug concentrations, drug-treated and untreated cells were labeled continuously with ¹⁴C-protein hydrolysate (UVVVR, Prague, Czechoslovakia; specific activity, 1 Ci/g) or [¹⁴C]phenylalanine (UVVVR; 270 mCi/mmol).

Rate of phage RNA synthesis. Samples of 0.5 ml of infected rifamycin-treated cultures were pulse-labeled at appropriate times for 2 min with [³H]uracil (V/O IZOTOP, Moscow, USSR; 6 Ci/mmol) at 50 μ Ci/ml as described elsewhere (1). To calculate the rate of RNA minus-strand synthesis, we determined the percentage of minus strands in pulse-labeled phage RNA at appropriate times by RNA-RNA hybridization (7), following the procedure described in reference 1. Radioactivity measurements were made with a Packard Tri-Carb 3380 liquid scintillation counter in toluene scintillation fluid with a [³H]RNA counting efficiency on paper disks of about 10%.

Rate of phage protein synthesis. Samples of 10 ml of infected rifamycin-treated cultures were pulse-labeled with ¹⁴C-protein hydrolysate at 1 μ Ci/ml. After

labeling, the cells were rapidly cooled in a dry-ice-acetone bath, collected by centrifugation, and lysed in 0.5 ml of buffer containing 0.01 M sodium phosphate (pH 6.9)-2% sodium dodecyl sulfate (SDS)-2% β -mercaptoethanol. The lysates were dialyzed by the polyacrylamide gel block method described by Greenlee (9) against 2×2 liters of buffer containing 0.01 M sodium phosphate (pH 6.9)-0.1% SDS. After dialysis, 0.5 ml of dialysis buffer and 5 μ l of β -mercaptoethanol were added to each sample. Samples (0.2 ml) were heated at 100°C for 3 min and applied to the polyacrylamide gels. SDS-disc gel electrophoresis was performed in 0.6- by 10-cm tubes according to the procedure described by Laemmli (15). The composition of the stacking and separating gels and electrode buffer were adopted from Maurer (16), except that all solutions contained 0.1% SDS. Gels were composed of three layers: (i) stacking gel, 1 cm—2.5% acrylamide-0.62% bisacrylamide-0.06 M Tris-0.03 M H₃PO₄-0.1% SDS (adjusted to pH 6.9); (ii) separating gel, 6 cm—10% acrylamide-0.4% bisacrylamide-0.375 M Tris-hydrochloride (pH 8.9)-0.1% SDS; (iii) separating gel, 2 cm—same as (ii), but 15% acrylamide-0.6% bisacrylamide. The electrode buffer contained 0.005 M Tris-0.04 M glycine-0.1% SDS (pH 8.6). Samples were concentrated within the stacking gel at 1 mA/tube, and separation was made at 5 mA/tube. Cytochrome *c* was used as an internal migration standard. At the end of the electrophoresis, the gels were sliced into 1-mm fragments. Each fragment was extracted with 0.4 ml of 0.5% SDS and subjected to the determination of radioactivity with Kinard scintillation fluid (counting efficiency, about 60%). Marker positions are shown in Fig. 1.

RESULTS

Synthesis of phage RNA and proteins during the replication cycle. We have studied the rates of RNA and protein synthesis during the phage replication cycle. To suppress macromolecular syntheses of the host, the drug rifamycin has been used. It was shown (1) that rifamycin had little effect on the main characteristics of phage replication and therefore may be helpful in elucidating the events of a single round of replication.

To determine the rates of synthesis of individual phage-specific proteins, 5-min pulse-labels with ¹⁴C-amino acids and subsequent polyacrylamide gel electrophoresis were performed. Figures 2 and 3 show typical examples of such electrophoregrams for *E. coli* strains AB259 (Su⁻) and H12R8a (Su-3), respectively, infected with MS2 and nonpolar coat protein amber mutant MS2*am623*. To determine the rates of the total phage RNA synthesis, cultures were pulse-labeled for 2 min with [³H]uracil. The percentage of minus strand in the phage pulse-labeled RNA was measured at appropriate times (1), and the values obtained were used to plot the kinetics of RNA minus-strand synthesis.

In Figure 4 are summarized the rates of syn-

FIG. 1. Marker positions in SDS-polyacrylamide disc gel electrophoresis. The protein bands were stained with Coomassie brilliant blue according to Laemmli (15). The markers are: (1) B' subunit of *E. coli* RNA polymerase; (2) β subunit of RNA polymerase; (3) σ subunit of RNA polymerase; (4) bovine serum albumin; (5) pyruvate kinase; (6) ovalbumin; (7) creatine phosphokinase; (8) aldolase; (9) α subunit of RNA polymerase; (10) chymotrypsinogen; (11) myoglobin; (12) cytochrome *c*. Arrows indicate the positions of phage proteins: replicase (R), A_s protein (A_s), A protein (A), and coat protein (C).

thesis found for viral macromolecules in nonsuppressor and suppressor strains of *E. coli* infected with wild-type phage MS2 and coat protein mutant MS2 $am623$. The maximum values of the total phage RNA synthesis are similar in all cases, which permits normalization of the corresponding rate curves. The other rate curves were normalized to enable comparison between cells infected with wild-type and coat protein mutant phage with respect to the amount of synthesis of viral macromolecules (see legend to Fig. 4). The rate curves for total RNA synthesis can be regarded at the same time as a fairly good representation of the kinetics of RNA plus-strand synthesis, since the plus-strand RNA accounts for approximately 90 and 80% of the overall RNA synthesized for MS2 and MS2 $am623$, respectively (see Table 1). The rate curves for RNA minus-strand synthesis in strain AB259 infected with MS2 and MS2 $am623$ are also shown. The rate curves for viral protein synthesis are depicted as stepped lines in the figure.

The decline in the replicase synthesis in all cases examined was found to be invariably followed by a reduction in the total phage RNA synthesis after a certain period of time. This period is rather prolonged in the RNase I-deficient Q13 strain. Production of RNA minus strands declines first, and the rate curves for replicase and minus-strand RNA synthesis become superimposed. This is true for both MS2- and MS2 $am623$ -infected cells, except that the decrease in MS2 $am623$ replicase and minus-strand synthesis begins 3 to 5 min later, the

observed delay resulting in a two- to fourfold replicase overproduction. Excessive synthesis of MS2 $am623$ replicase is accompanied by a similar overproduction of minus-strand, but not of plus-strand, RNA (Table 1).

As expected, the rate curve for the coat protein synthesis in MS2-infected cells was displaced with respect to the RNA rate curve further to the right along the abscissa. The synthesis of the coat protein amber fragments in the MS2 $am623$ -infected cells was not detected due, presumably, to their low production, which is in agreement with the findings of others (21, 27). The synthesis of nonfunctional MS2 $am623$ coat protein found in Su-3 cells inserting tyrosine instead of glutamine was also low, as shown earlier (21). Somewhat surprising was the low level of the functional MS2 $am623$ coat protein synthesis in Su-1 cells inserting serine (see Discussion).

The rate curves for A protein synthesis represent sums of values for both A protein and readthrough protein A_s , the latter appearing only in suppressor strains. It is evident that the coat protein mutant makes less A protein as compared with wild-type phage. As can be seen in the electrophoregrams in Fig. 3, the protein found between replicase and A protein may, in fact, be identical to the A_s protein described by Remaut and Fiers (20). This is confirmed by the findings that this protein is not synthesized by the A protein mutant MS2 $am302$ in nonpermissive strains whereas in permissive strains its synthesis is markedly reduced (not shown). According to our electrophoregrams, A_s protein has

FIG. 2. Polyacrylamide gel electrophoresis of ^{14}C -labeled proteins from MS2- and MS2am623-infected *E. coli* AB259 (Su^-). Rifamycin was added at 3 min postinfection. Proteins migrated from left to right. Arrows indicate the peak positions of replicase (R), A_s protein (A_s), A protein (A), and coat protein (C). The numbers on the electrophoregrams signify the pulse time (minutes postinfection). Protein synthesis in rifamycin-treated uninfected cells (from 15 to 20 min relative to the time of infection) is shown by the broken line.

a molecular weight of about 50,000. Figure 5 shows that the proportion of A_s protein in the total ($A + A_s$) protein synthesis is small at the early stages of the wild-type phage replication, but it gradually approaches its maximum value, which corresponds to the level of suppression in Su-1 and Su-3 cells, 30 and 50%, respectively (8). The coat protein mutant MS2am623 in Su-3 cells has low A_s protein synthesis throughout replication. MS2am623 in Su-1 cells is characterized by A_s protein values intermediate between wild-type phage in Su-1 and MS2am623 in Su-3 cells.

The pattern of replicase and RNA synthesis is actually the same in fr, R17, f2, Q β , and MS2am302, an A protein mutant, if compared

with MS2. No differences whatever have been found for replicase and RNA synthesis in MS2am601, a coat protein mutant, and f2sus11, a double mutant for both A protein and coat protein, when compared with MS2am623 (not shown). Replicase is the only complete phage protein synthesized in the f2sus11-infected non-permissive host; nevertheless, its synthesis declines in the same manner as demonstrated in Fig. 4 for MS2am623.

Synthesis of phage RNA and replicase after suppression of protein synthesis. Suppression of protein synthesis disturbs the normal course of events during the replication cycle, which results in the prolongation of phage RNA synthesis (18, 22). The observed effect is pro-

FIG. 3. Polyacrylamide gel electrophoresis of ^{14}C -labeled proteins from MS2 and MS2am623-infected *E. coli* H12R8a (Su-3). Symbols and abbreviations are the same as for Fig. 2.

duced using different protein inhibition procedures: chloramphenicol, tetracycline, and streptomycin treatment or valine-isoleucine restriction (Fig. 6). In each case the presence of rifamycin is a prerequisite for observation of the prolongation effect (19).

Figure 7 demonstrates the significance of the time (relative to the time of infection) when protein synthesis is inhibited. The treatment of infected cells with chloramphenicol (50 $\mu\text{g}/\text{ml}$) late in the infection, at 25 or 35 min postinfection, fails to produce effective prolongation of RNA synthesis, whereas addition of the drug at 15 min does.

Correlation between the pattern of RNA synthesis and the level of residual protein synthesis (Fig. 8) was found to be the most essential feature of the prolongation effect. At low inhibition values, RNA synthesis declines, although with a delay (for example, in the presence of 2 μg of chloramphenicol per ml). When inhibition

of protein synthesis reaches 60 to 90%, which can be achieved by applying 10, 25, and 50 μg of chloramphenicol per ml, maximum prolongation of RNA synthesis is observed. Inhibition of protein synthesis by more than 99%, caused by 200 μg of tetracycline per ml, results in an immediate decrease in RNA synthesis. A similar effect of inhibitors of protein synthesis on viral RNA accumulation was observed in all bacteriophages under study, including A protein, coat protein, and double (A and coat) amber mutants (not shown).

In Figure 9 are depicted the rates of replicase and RNA minus-strand synthesis in MS2- and MS2am623-infected cells after exposure to chloramphenicol in concentrations allowing prolonged RNA synthesis. Although chloramphenicol inhibits protein synthesis by 92%, as estimated from the synthesis of coat and A proteins, the rate of replicase synthesis is reduced only twofold (MS2-infected cells) or fourfold

FIG. 4. MS2 and MS2am623 RNA and protein synthesis in different strains of *E. coli*. Rifamycin was added at 3 min postinfection. All rate curves are normalized. For the total phage RNA synthesis, the maximal rate in each case is set at 1; for minus strands and replicase, the maximal rate for the indicated strain infected with MS2am623 is set at 1; for coat protein and A protein, the maximal rate for the indicated strain infected with MS2 is set at 1. The rate curves for protein synthesis are shown as stepped lines: solid, replicase; broken, coat protein; dotted, A protein. The rate curves for RNA synthesis are shown with the symbols: (○) total RNA; (●) RNA minus strands.

TABLE 1. Molecules^a of RNA plus and minus strands and viral proteins per infective center of phage MS2 and MS2am623 in rifamycin-treated *E. coli* AB259 (Su⁻)

Parameter	MS2 (1)	MS2am- 623 (2)	Ratio (2)/(1)
RNA plus strands	30,000	23,000	0.8
RNA minus strands	3,300	6,000	1.8
Replicase	6,200	20,000	3.1
Coat protein	1,500,000		

^a To calculate the number of phage macromolecules synthesized from 10 to 28 min postinfection, the corresponding rate curves were integrated assuming that [³H]uracil is incorporated into phage RNA without appreciable dilution of the label and that ¹⁴C-labeled amino acids are diluted approximately 50-fold under the conditions used. This value was obtained by measuring in a parallel experiment the ¹⁴C-to-³H ratio within the purified (in a CsCl gradient) phage particles double-labeled with [³H]uracil and ¹⁴C-amino acids. The specific activity of phage [³H]RNA was known. Rifamycin was added at 3 min postinfection. The number of infective centers was assumed to be 10⁸ PFU/ml.

FIG. 5. Percentage of A_s protein in the total ($A + A_s$) protein synthesis in MS2- and MS2am623-infected *E. coli* S26R1E (Su-1) and H12R8a (Su-3) cells.

(MS2am623-infected cells). Moreover, chloramphenicol treatment maintains the synthesis of replicase at an appreciable rate for a certain period of time in both MS2- and MS2am623-infected cells. This results in the overproduction of replicase in chloramphenicol-treated cells in comparison with the untreated control, as indicated in Table 2. Figure 9 shows also that in both cases the rate curve of minus-strand RNA synthesis invariably follows that of replicase syn-

thesis after chloramphenicol treatment. The data presented in Table 2 indicate that besides temporal coincidence of their rate curves, RNA minus strands and replicase are synthesized in nearly equimolar amounts in the presence of chloramphenicol.

DISCUSSION

The fact that replicase and RNA syntheses are coupled becomes apparent from the data

FIG. 6. MS2 RNA synthesis in *E. coli* AB259 with protein synthesis inhibited at 15 min postinfection. Rifamycin was added at 10 min postinfection. Symbols: (○) control without protein synthesis inhibitors; (●) with chloramphenicol, 50 μ g/ml; (Δ) with valine, 500 μ g/ml; ($\blacktriangle$) with streptomycin, 50 μ g/ml; (∇) with tetracycline, 10 μ g/ml.

FIG. 7. Rate of MS2 RNA synthesis in *E. coli* AB259 treated with chloramphenicol at different times. Rifamycin was added at 3 min postinfection. Chloramphenicol, 50 μ g/ml, was added (●) 15, (Δ) 25, or ($\blacktriangle$) 35 min after infection. (○) Control without chloramphenicol.

FIG. 8. Rate of MS2 RNA synthesis (A) and accumulation of viral proteins (B) in *E. coli* AB259 at different concentrations of protein synthesis inhibitors. Rifamycin was added at 10 min, chloramphenicol or tetracycline was added at 15 min, and [^{14}C]phenylalanine was added at 17 min postinfection. Chloramphenicol concentrations were: (●) 2 $\mu\text{g/ml}$; (Δ) 10 $\mu\text{g/ml}$; ($\blacktriangle$) 25 $\mu\text{g/ml}$; (∇) 50 $\mu\text{g/ml}$; ($\blacktriangledown$) 200 $\mu\text{g/ml}$. Tetracycline concentration was ($\square$) 200 $\mu\text{g/ml}$. (○) Control without protein synthesis inhibitors.

obtained in the study of normal replication of wild-type phage (see Fig. 4). Further evidence is provided by the experiments, in which substantial changes in the pattern of replicase synthesis have been elicited. These are the experiments performed on cells infected with the wild-type phage using inhibitors of protein synthesis. The effect of such inhibitors on viral RNA replication is phage specific because it can be produced irrespective of the mode of protein synthesis inhibition (as seen in Fig. 6), thus ruling out the participation of host regulators, such as the *rel* gene product, in the control of phage RNA synthesis. Strong dependence of RNA replication on the continuous production of replicase is shown by the fact that the rate of RNA synthesis drops off after complete blocking of protein synthesis (see Fig. 8). For the prolongation of RNA synthesis, (i) protein synthesis should be suppressed before the repressor of replicase synthesis, i.e., the coat protein, has been accumulated (Fig. 7), and (ii) inhibition of protein synthesis must be effective enough to block rapid accumulation of the repressor (Fig. 8), allowing replicase synthesis at the same time (Fig. 9).

RNA minus strands, after exposure to chloramphenicol, are synthesized at a nearly constant rate following the pattern of replicase formation (Fig. 9). Furthermore, Table 2 indicates that both replicase and minus strands are produced in nearly equimolar amounts. The four- to five-

fold overproduction of replicase and minus strands occurs in chloramphenicol-treated cells as opposed to untreated control, whereas plus strands are overproduced only 2.5-fold.

The pattern of replicase synthesis becomes altered also in cells infected with the nonpolar coat protein amber mutants. The peak of MS2*am*623 replicase synthesis is observed later in the replication cycle, which results in replicase overproduction (see Fig. 4). RNA minus strands display similar changes in their accumulation rates and are overproduced equally with replicase, whereas production of plus strands is even reduced as compared with replication of wild-type phage (see Table 1).

Thus, it is evident that the changes in the pattern of replicase synthesis in both cases reported above are followed primarily by similar changes in the synthesis of RNA minus strands, suggesting possible coupling of both processes. In principle, such a mode of control of RNA accumulation is possible if translation of the replicase cistron is coupled with the RNA minus-strand initiation and RNA minus strands are functionally unstable. As to the latter assumption, it is safe to claim that viral RNA minus strands, as well as unencapsidated plus strands, are degraded in analogy with the host mRNA. This is supported by the data demonstrating the breakdown of parental RNA to nucleotides as soon as 10 to 15 min after infection (3). Elonga-

tion of the lifetime of RNA minus strands, which possibly takes place in RNase-deficient strains such as Q13 and AB105, may be able to increase plus-strand synthesis by one minus-strand template. This would result in protracted replication cycles in these strains (Fig. 4; 1).

If our assumption is valid, the mechanism of coupling of minus-strand synthesis with repli-

FIG. 9. MS2 and MS2am623 replicase and RNA minus-strand synthesis in *E. coli* (Su^-) within the replication cycle and after exposure to chloramphenicol. Rifamycin was added at 3 min and chloramphenicol (50 $\mu\text{g}/\text{ml}$) was added at 15 min postinfection. The rate curves for replicase are shown as stepped lines: solid, without chloramphenicol; broken, with chloramphenicol. Symbols: (○) RNA minus strands without chloramphenicol; (●) RNA minus strands after chloramphenicol addition.

case synthesis should consist in making accessible the 3'-end and/or some other parts of the plus-strand templates for initiation of RNA minus-strand synthesis. This accessibility is presumably secured by the ribosome movement along the replicase cistron during its translation, which unfolds RNA macrostructure and exposes the replicase binding sites. This probably explains why the partially purified f2 replicase synthesizes almost exclusively RNA plus strands on the f2 replicative ensemble as template (5, 6). Pure Q β replicase is able, however, to produce in vitro equivalent amounts of plus and minus strands (which is not found in vivo) but only in the presence of host factor (11, 14), modifying the macrostructure of plus templates (23). Host factor does not stimulate the activity of pure f2 replicase (6), and its participation in phage replication in vivo has not been established so far.

If RNA replication can be controlled by switching off replicase synthesis, a question arises as to the actual mechanism of the latter. It is known that during wild-type phage replication replicase synthesis is governed by the coat protein through repressor complex I (for references see 10, 32). The clue to the question why replicase and subsequently RNA syntheses decline in the coat protein mutants is provided by the experiments in which protein synthesis is partially inhibited (see Fig. 9, bottom). It appears that replicase production is repressed late in the replication cycle of coat protein mutants and becomes restored when inhibitors of protein synthesis are added. Since similar effects also occur in nonsuppressor cells infected with f2sus11, where replicase is the only complete viral protein, one can assume that replicase itself may inhibit its own synthesis on excessive accumulation.

A model of replicase-controlled regulation for coat protein and replicase synthesis on Q β RNA, by means of repressor complex II, has been put forward by Kolakofsky and Weissmann (12, 13). According to this model, the function of repres-

TABLE 2. Molecules^a of RNA plus and minus strands and viral proteins per infective center of phage MS2 and MS2am623 in rifamycin-treated *E. coli* AB259 after exposure to chloramphenicol

Parameter	MS2			MS2am623		
	Without chloramphenicol (1)	With chloramphenicol (2)	Ratio (2)/(1)	Without chloramphenicol (3)	With chloramphenicol (4)	Ratio (4)/(3)
RNA plus strands	17,500	44,000	2.5	17,000	43,000	2.5
RNA minus strands	1,000	4,600	4.6	2,200	10,000	4.5
Replicase	900	4,000	4.5	2,000	8,500	4.3
Coat protein	1,500,000	125,000	0.08			

^a To calculate the number of phage macromolecules synthesized from 20 to 50 min postinfection, the corresponding rate curves were integrated. Rifamycin was added at 3 min, and chloramphenicol (50 $\mu\text{g}/\text{ml}$) was added at 15 min postinfection. For other details, see footnote to Table 1.

sor complex II consists in removing ribosomes from the plus-strand templates and switching the latter from translation to replication. Our findings demonstrate that repressor complex II is indeed functional *in vivo* and that replicase in this complex does bind to the coat protein initiation site on plus templates, as shown *in vitro* for Q β replicase and Q β RNA (17, 30, 31). In fact, excess replicase existing at early stages of wild-type replication or throughout the cycle of coat protein mutant replication is invariably accompanied not only by increased RNA minus-strand synthesis, but also by the decreased synthesis of coat protein (Fig. 4) or its amber fragments (21, 27) and protein A_s (Fig. 5).

Decrease in the total RNA synthesis in the cells infected with the coat protein mutant indicates that transformation of repressor complex II into the initiation complex for minus-strand synthesis postulated by Kolakofsky and Weissmann (12, 13) may be limited under certain conditions, viz., in the case when the replicase cistron is not occupied by ribosomes. Early in wild-type phage replication, when the molar ratio of ribosomes to RNA is relatively high and the absolute amounts of replicase appear to be low, repressor complex II transforms readily into initiation complex. On the contrary, in cells infected with coat protein mutants having excessive replicase synthesis, the ratio of replicase to RNA is increased at any stage of replication. This increase is accompanied by a permanent drop in the ribosome-to-RNA ratio since accumulation of the RNA plus strands fails to produce phage particles. Therefore, the number of ribosome-free RNA plus strands involved in repressor complex II and unable to initiate RNA minus-strand synthesis rises, especially at late stages of replication, thereby shutting off any further RNA synthesis.

A special explanation should be reserved for the low values of coat protein synthesis in Su-1 cells infected with the coat protein mutant. Rifamycin, abolishing competition of viral replication with cellular processes (19), stimulates extensive synthesis of replicase, the coat protein synthesis being partially suppressed (ca. 30% of the wild-type level [8]). This results in the enhanced formation of repressor complex II not observed in growing, rifamycin-free cultures.

Consequently, the mechanism controlling translation of phage templates appears at the same time to be responsible for the regulation of viral RNA replication, since RNA synthesis and translation of replicase cistron are found to be coupled. Such a regulatory mechanism seems reasonable with respect to the efficiency of phage production. To test the proposed hypoth-

esis, both experimental and mathematical modeling would be welcome.

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