Effect of the "RNA Control" Locus in *Escherichia coli* on RNA Bacteriophage R23 Replication

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The effect of the *rel* gene of *Escherichia coli* on the RNA synthesis induced by phage R23 was studied. This RNA phage has the property of inhibiting ribosomal RNA formation and completely dominating the RNA synthesis of the host. Phage-specific RNA formation was found to be dependent on the allelic state of the *rel* gene. Determinations of RNA synthesis were made by both cumulative and short-term incorporations of uracil and adenine. Variations in labeling of nucleotide pools were compensated for by determining specific activities of ATP and UTP and using these values to obtain true, relative rates of RNA synthesis.

Rapid synthesis of RNA in bacteria is dependent on the presence of amino acids. Amino acid regulation of RNA synthesis is a well-established phenomenon and has been shown to be governed by the *rel* locus of *Escherichia coli* (for review see reference 5). Full synthesis of RNA is dependent not only on the presence of free amino acids but also on a full complement of aminoacyl-tRNA (15). During amino acid (or aminoacyl-tRNA) deprivation in a *rel*⁺ strain, rRNA synthesis is rapidly curtailed and a concomitant accumulation of the two guanosine nucleotides ppGpp and pppGpp is observed (11). Under similar conditions in a *rel*⁻ strain, rRNA synthesis continues unabated, and neither ppGpp nor pppGpp is formed.

The mechanism of amino acid regulation as it works at the transcriptional level in vivo is unknown, although a direct effect on the activity of the DNA-dependent RNA polymerase would be conceivable. To test such an idea it would be of interest to investigate whether the *rel* gene, which is directly linked to ppGpp synthesis in *E. coli*, also governs the RNA synthesis that is effected by bacteriophage-specific, RNA-dependent RNA polymerase. An ideal tool for this investigation would be an RNA phage with the ability to turn off ribosomal RNA synthesis, because in such a system phage-specific RNA synthesis could easily be separated from host-specific RNA synthesis. Phage R23, which resembles f2 in chemical, physical, and immunological characteristics, has been reported to dominate the RNA synthesis of the host cell completely (22).

In the present investigation phage R23 was shown by electrophoretic analysis to be able to

turn off completely the rRNA synthesis of the host. The formation of R23 phage RNA was furthermore found to be dependent on the presence of free amino acids. This stringent regulation of phage RNA synthesis was dependent on the allelic state of the *rel* locus of the host and was shown to be absent in a *rel*⁻ strain.

MATERIALS AND METHODS

Phage and bacteria. The strains of *E. coli* K-12, Cp78 (*rel*⁺) and Cp83 (*rel*⁻), were obtained from N. Fiil. They were originally derived from *E. coli* K-12 W677, are F⁻, require arginine, histidine, leucine, threonine, and thiamine, and are isogenic except at the *rel* locus (6). The described strains were made F⁺ and susceptible to RNA phage infection by incubation with a prototrophic male strain of *E. coli* K-12. The male donor was mixed with the auxotrophic F⁻ strain in equal proportions at a total cell concentration of about 0.5×10^9 /ml in a rich medium (supplemented LB of Nordström et al. [17]). After incubation at 37 C for 60 min without shaking, the cell mixture was plated and the small colonies of auxotrophic bacteria were picked and tested for the male property by RNA phage susceptibility. The relaxed and stringent RNA synthesis patterns for the male strains constructed in this way are shown in Fig. 1, where the *rel*⁻ phenotype is clearly discernible by the cumulative incorporation of radioactive adenine into acid-insoluble material. *E. coli* Q13 (10) was used routinely for the assay of phage. RNA bacteriophage R23 (22) was supplied by J. T. August. Phage stocks were prepared according to Watanabe and August (21).

Chemicals. [2-¹⁴C]uracil (60 or 22 mCi/mmol) and [8-¹⁴C]adenine (60 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, England, or New England Nuclear Chemicals, Dreieichenhain, Germany. [5-³H]uracil (20 Ci/mmol) and [2-³H]adenine (18 Ci/mmol) were obtained from The Radiochemical Centre. [³²P]orthophosphate was purchased

FIG. 1. Incorporation of [^{14}C]adenine into acid-insoluble product under amino acid starvation of the used F^+ strains with and without phage R23 infection. At -60 min, cells were diluted into the low-phosphate mineral salts medium supplemented with [^{14}C]adenine ($0.2 \mu\text{Ci/ml}$, $6 \mu\text{g/ml}$) and incubated at 37°C without aeration for 60 min. At time zero, viable count determination (1.5×10^8 cells/ml) and phage infection took place. Aeration was started at 15 min. Amino acids were removed by filtration at 40 min and added back at 80 min. Samples of 0.1 ml were withdrawn and precipitated on filter paper disks as described in the text. Values along the ordinate represent counts per minute incorporated per 0.1 -ml sample. Symbols: $\times$, Cp78 F^+ ; $\blacktriangle$, Cp78 F^+ + R23; $\bullet$, Cp83 F^+ ; $\circ$, Cp83 F^+ + R23.

from AB Atomenergi, Studsvik, Sweden. Amino acids, thiamine, uracil, and adenine were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Polyethylene glycol (A 6000) and sodium dextran sulfate (500) for phage purification were purchased from Union Carbide Corp., New York, and Pharmacia, Uppsala, Sweden, respectively. Acrylamide (catalogue no. 5521, for electrophoresis), N,N' -methylenebisacrylamide, and N,N,N',N' -tetramethylethylenediamine were the products of Eastman Kodak Co., Rochester, N.Y. Agarose was purchased from Miles-Seravac, Maidenhead, Berks., England. Cellulose thin-layer plates (cellulose polyethylenimine MN Polygram cellulose 300 PEI) were obtained from Machery Nagel and Co., Büren, West Germany. The plates were soaked for 30 min in distilled water and dried before use.

Media. Most experiments were carried out in a

low-phosphate mineral salts medium of the following composition (per liter): 0.5 g of NH_4Cl , 0.5 g of $(\text{NH}_4)_2\text{SO}_4$, 5.0 g of NaCl , 0.5 g of KCl , 0.203 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 14.7 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.178 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, and 6.25 g of Tris. pH was adjusted to 8.0 with 1 M HCl and, after autoclaving, the medium was supplemented with 0.9 ml of 1 M CaCl_2 /liter, glucose to 5 g/liter, and FeCl_3 to 10^{-6} M. Supplements were amino acids ($50 \mu\text{g/ml}$) and thiamine ($1 \mu\text{g/ml}$). Media for plating of phage R23 were as described by Watanabe and August (21).

Infected cells. An overnight culture was diluted to about 1.5×10^8 cells/ml in the low-phosphate mineral salts medium supplemented as necessary. The culture was then left for 60 min at 37°C without aeration before the addition of phage. This procedure was found to give good and reproducible phage production. At time zero phage was added to 20 to 30 PFU/bacterium. Aeration was started at 15 min after infection. Amino acid starvation was initiated by filtration on a membrane filter (Gelman, Metricel GA-6) and resuspension in amino acid-deficient medium. Phage production was determined routinely and amounted to $1,200$ to $1,800$ phages/bacterium. This burst size concurs with that reported by Lodish and Zinder (14) for phage f2 in *E. coli* K-12.

Determination of RNA synthesis. The synthesis of RNA was measured by the cumulative incorporation of [^{14}C]uracil or [^{14}C]adenine into acid-insoluble product. Determination of RNA synthesis was performed either by precipitating a 1 -ml sample of the labeled culture in 5 ml of cold 5% trichloroacetic acid and collecting the precipitate on a glass filter (Whatman GF/C) or by pipetting a 0.1 -ml sample onto a 2 -cm-diameter filter paper disk, which was immediately immersed into cold 5% trichloroacetic acid (1). Filters were dried and then counted in a Packard scintillation counter.

Determination of the relative rate of RNA synthesis. Relative rates of RNA synthesis were determined mainly as described by Winslow and Lazzarini (24). To measure the relative specific activity of the ATP and UTP pools, bacteria were labeled for several generations with [^{32}P]orthophosphate (10 to $15 \mu\text{Ci/ml}$, 10 to $15 \text{ Ci}/\mu\text{mol}$). Labeled bacteria were diluted into low-phosphate mineral salts medium (containing [^{32}P]orthophosphate at the same specific activity [10 to $15 \mu\text{Ci}/\mu\text{mol}$]) and incubated at 37°C for 60 min without aeration, to yield about 1.5×10^8 cells/ml (cf. infected cells above and Fig. 1). The cells were then infected with R23, and aeration was started 15 min later. At indicated intervals 500 - μl samples of the culture were withdrawn and added to vials containing either [2 - ^3H]adenine (final concentration $20 \mu\text{Ci/ml}$, $20 \text{ mCi}/\mu\text{mol}$) or [5 - ^3H]uracil ($20 \mu\text{Ci/ml}$, $20 \text{ mCi}/\mu\text{mol}$).

After 60 s of incubation at 37°C under aeration, a 100 - μl sample was withdrawn and pipetted into $50 \mu\text{l}$ of 4 M formic acid in an ice bath. After 30 min of extraction at 0°C , samples were centrifuged for 15 min at $10,000 \times g$, and $10 \mu\text{l}$ of the supernatant was applied to a polyethylenimine cellulose thin-layer plate. As references, UTP and ATP ($0.1 \mu\text{mol}$ of each)

were added. The chromatograms were run up twice with water before development in 0.75 M KH_2PO_4 , pH 3.4 (3).

The ATP and UTP spots were located under UV or by autoradiography, cut out, and placed in a scintillation vial. The nucleotide was then extracted with 250 μl of 2 M NH_4OH for 20 min and finally counted in 5 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) scintillation liquid containing 5% acetic acid and 8% water to decrease chemiluminescence. The settings of the scintillation spectrometer used gave 7% spillover of ^{32}P counts into the ^3H channel.

Determinations of guanosine tetraphosphate were performed in a similar way and mainly as described earlier (3).

The rate of incorporation of [^3H]adenine or [^3H]uracil into RNA was determined in cultures treated exactly as described above except that they were not labeled with ^{32}P . At intervals of 15 s, 50- μl samples were removed from the 500- μl culture samples and pipetted into 2.0 ml of 0.3 M NaOH in an ice bath. One half of each sample was then neutralized with 0.3 M HCl and precipitated with 5% trichloroacetic acid. The other half of each sample was incubated at 37 C for 12 h, cooled, neutralized with 0.3 M HCl, and finally precipitated with 5% acid. The precipitates were collected on membrane filters (Sartorius, Göttingen, Germany), which were dried and then counted in the scintillation counter. The counts remaining after alkaline hydrolysis were subtracted from total trichloroacetic acid-precipitable counts to give incorporation into RNA. This value was then divided by the relative specific activity ($^3\text{H}/^{32}\text{P}$ ratio), at the corresponding time interval, of the ATP and UTP pools, respectively, to give the relative rate of RNA synthesis.

Extraction of RNA. RNA for electrophoretic analysis was extracted from 5-ml samples withdrawn from the aerated, phage-infected cultures. The infected bacteria were rapidly centrifuged, resuspended in 3 ml of electrophoresis buffer, quickly frozen, and disintegrated in a high-pressure cell (X-press, AB Biotec, Stockholm, Sweden). To the disintegrated material was added 0.1 ml of a bentonite suspension (30 mg/ml), fractionated according to Fraenkel-Conrat et al. (8). After thawing, the suspension was centrifuged for 15 min at 0 C and $20,000 \times g$. To the supernatant was added 0.1 ml of bentonite suspension (30 mg/ml) and 0.07 ml of carrier RNA solution (2 mg/ml) prepared from uninfected *E. coli* Cp78 F⁻. RNA was then extracted by the method of Peacock and Dingman (18) for cytoplasmic RNA. After treatment with 0.1% sodium dodecyl sulfate for 4 min at room temperature, the supernatant was mixed for 15 min with an equal volume of water-saturated phenol. Sodium acetate was added to the mixture to a final concentration of 0.3 M. The separated phenol phase was reextracted for 5 min with 0.5 ml of electrophoresis buffer. The combined water phases were reextracted once with 0.5 volume of phenol for 5 min, and RNA was finally precipitated from the separated water phase with 2 volumes of cold ethanol at -20 C overnight. After centrifugation, the precipitated RNA

was washed as described by Peacock and Dingman (19) and finally dissolved in 0.5 ml of electrophoresis buffer. RNA concentration was determined by UV absorption, using an extinction coefficient at 260 nm of 0.24 for a 0.001% RNA concentration.

Acrylamide gel electrophoresis. The procedure for the preparation of the gels and the electrophoretic techniques were mainly those of Peacock and Dingman (18). The electrophoresis apparatus used was that originally described by Davis (4). A composite gel of 0.5% agarose and 2.5% acrylamide was used throughout. The electrophoresis buffer was Tris-chloride (0.025 M), pH 8.0-MgCl₂ (0.001 M). All gels were prerun for 1 h at 9 mA/tube, the gels were cooled, and the buffer was changed before application of the samples, which consisted of 10 μl of RNA (about 0.5 mg/ml) and 20 μl of 24% glycerol, with added bromophenol blue as a dye marker. All samples were electrophoresed for 2 h at 9 mA/tube. After electrophoresis the gels were soaked in 1 M acetic acid for 15 min and then stained for 20 min in 0.2% methylene blue in 0.4 M sodium acetate buffer, pH 4.7. The excess stain was removed by rinsing in water. To measure radioactivity, the gels were cut into slices, 1.40 mm thick, each of which was transferred to a scintillation vial and treated with 0.5 ml of 0.5 M NH_4OH for 2 h at 80 C. The samples were then dried and finally counted in the scintillation counter after the addition of 10 ml of naphthalene dioxane scintillation fluid.

RESULTS

Growth characteristics for phage R23 in the bacterial strains used. Production of R23 in the stringent male strain Cp78 F⁺ is demonstrated in Fig. 2A. At 37 C and in the low-phosphate mineral salts medium used, mature phage began to appear intracellularly at 50 to 60 min after infection. A burst size of 1,800 phages/bacterium was reached within 120 min. The inability of the original female strain Cp78 F⁻ to support phage growth is demonstrated in the same figure for comparison. Phage R23 had the ability to kill its host. This is shown in Fig. 2B, where the exponential killing of the male strains of Cp78 and Cp83 is demonstrated. The percentage of surviving bacteria was 50% at 25 to 30 min after infection and decreased to 5% at 120 min. It is shown for comparison that the female strain of Cp78 was completely unaffected by phage.

Interference of R23 with host RNA synthesis. The interference of phage R23 with host growth is also reflected in the curves representing RNA synthesis (Fig. 3). The upper curve demonstrates RNA synthesis after the infection of Cp78 F⁻ with R23. The cumulative incorporation of [^{14}C]uracil increased exponentially in the growing, phage-insensitive cells. In the sensitive Cp78 F⁺ cells, however, RNA synthe-

FIG. 2. Growth characteristics of R23 in Cp78 F⁺, Cp78 F⁻, and Cp83 F⁺. (A) Cells were grown and infected at 0.5×10^8 cells/ml in the low-phosphate mineral salts medium as described in the text. At the times indicated, 0.5-ml samples were withdrawn and lysed as described (22). Intracellular phage production expressed as phages per bacterium was plotted versus time. Symbols: O, Cp78 F⁺; □, Cp78 F⁻. (B) Cells were grown and infected as in (A) but at 2.5×10^8 cells/ml. At times indicated, samples were withdrawn and titrated by viable count of bacteria. The fraction of surviving bacteria is plotted versus time on a semilog scale. Symbols: O, Cp78 F⁺, ×, Cp78 F⁻; Δ, Cp83 F⁺.

FIG. 3. Synthesis of RNA in Cp78 F⁺ after R23 phage infection. Cells were grown and infected at 0.5×10^8 cells/ml in the low-phosphate mineral salts medium supplemented with [¹⁴C]uracil (0.1 μCi/ml, 10 μg/ml). Samples of 0.1 ml were withdrawn and precipitated on filter paper disks as described in the text. Values along the ordinate represent counts per minute incorporated per 0.1-ml sample. Symbols: ×, Cp78 F⁻; O, Cp78 F⁺; Δ, Cp78 F⁺ + chloramphenicol (20 μg/ml) added at 5 min after infection.

sis diminished but not until about 80 min after infection, i.e., at a time when 80 to 90% of the cells were already killed by phage (cf. Fig. 2B). The lower curve for comparison shows the RNA synthesis in R23-infected Cp78 F⁺ cells, in which protein synthesis was inhibited by the

addition of chloramphenicol at 5 min after infection and the phage could thus not establish its RNA synthesis. The middle curve, demonstrating RNA synthesis after normal phage infection, then seems to show that whatever amount of host-specific RNA synthesis was turned off by phage, during the first 80 min of infection, was substituted by phage-specific RNA synthesis, because the cumulative [¹⁴C]uracil incorporation in that time period was identical in the F⁻ and F⁺ strains.

The fraction of ribosomal and phage-specific RNA synthesis, respectively, was determined by electrophoretic analysis on polyacrylamide gels. The RNA synthesis pattern obtained in this way for F⁺ and F⁻ bacteria is shown in Fig. 4. When [¹⁴C]uracil was added to Cp78 F⁻ cells at 40 min after the addition of phage, the incorporation of radioactivity between 40 and 60 min was predominantly into ribosomal 16S and 23S RNA (Fig. 4A). In the corresponding experiment with the F⁺ strain, the typical rRNA labeling pattern was abolished (Fig. 4B), and instead a more diffuse labeling pattern occurred.

A new peak, running in front of the 23S marker, was observed when the [¹⁴C]uracil incorporation took place between 30 and 90 min after infection (Fig. 4C) and ought to represent R23 phage-specific RNA, since it partly coincided with labeled RNA extracted from purified, mature phage (Fig. 4D).

Effect of the rel locus of the host on R23-

FIG. 4. Electrophoretic analysis of host and phage RNA synthesis on polyacrylamide gels. *Cp78 F⁻* and *Cp78 F⁺* cells were grown and infected at 2.5×10^8 cells/ml in the low-phosphate mineral salts medium to which uracil (2 μ g/ml) was added at 20 min and [14 C]uracil (0.1 μ Ci/ml) was added at different times after infection. Samples of 5 ml were withdrawn, RNA extracted, and subjected to electrophoresis as described in the text. Labeled R23 RNA was prepared from phage grown in *E. coli* K38 (21) in the presence of [14 C]uracil (0.1 μ Ci/ml, 2 μ g/ml) and purified as described. The labeled phage suspension was mixed with unlabeled phage before RNA extraction. The final activity was about 500 counts/min per 10^{11} PFU of phage. Extraction and electrophoresis of RNA was performed as described for the infected cells. The values along the ordinates represent radioactivity per gel slice, the numbers of which are plotted along the abscissae. Below each frame the positions of the stained bands are shown. Gels A, B, and C were run for 2 h, and gel D was run for 4 h. (A) *Cp78 F⁻*, labeled for 40 to 60 min after infection; (B) *Cp78 F⁺*, labeled for 40 to 60 min after infection; (C) *Cp78 F⁺*, labeled for 30 to 90 min after infection; (D) R23 RNA, run together with unlabeled RNA from uninfected *Cp78 F⁺*.

specific RNA synthesis. The effect of amino acid starvation on the RNA synthesis in *Cp78 F⁺* and *Cp83 F⁺* infected with phage R23 is shown in Fig. 5. In the stringent strain, [14 C]uracil, when added at 3 min after amino acid removal (40 min after infection), was to

some extent incorporated into acid-insoluble material. This incorporation took place at a rate that was only about 2.5% of that obtained after readdition of amino acids. When the identical experiment was performed with [14 C]adenine as the radioactive precursor, the rate of in-

FIG. 5. Incorporation of [^{14}C]uracil or [^{14}C]adenine into the RNA of stringent and relaxed host strains after R23 phage infection. Cells of Cp78 F^+ , Cp78 F^- , and Cp83 F^+ were grown and infected as described in Fig. 1. Amino acids were removed by filtration at 40 min after infection, and 3 min later [^{14}C]uracil or [^{14}C]adenine ($0.1 \mu\text{Ci/ml}$, $4 \mu\text{g/ml}$) was added. RNA was determined in 1-ml samples as described in the text. Amino acids were added back at 80 min after infection. Symbols: $\times$, Cp78 F^+ + R23, [^{14}C]uracil; $\blacktriangle$, Cp78 F^+ uninfected, [^{14}C]adenine; $\bullet$, Cp78 F^+ + R23, [^{14}C]adenine; $\blacksquare$, Cp83 F^+ + R23, [^{14}C]uracil; $\circ$, Cp83 F^+ + R23, [^{14}C]adenine.

corporation without amino acids was about 13% of that seen after the end of starvation. Figure 5 also describes similar experiments with Cp83 F^+ , where no effect of amino acid starvation on either uracil or adenine incorporation was observable. The experiments of Fig. 5 then seem to show that at 40 min after R23 infection, when according to Fig. 4 most RNA synthesis is phage specific, amino acid deprivation of the stringent strain severely restricted the incorporation of both uracil and adenine into RNA. The demonstrated difference in uptake between uracil and adenine agrees with observations by Lazzarini and Dahlberg (13), who observed the uptake of adenine into ATP to be much less affected than that of uracil into UTP under stringent conditions. The restriction of radioactive precursor incorporation into R23 RNA

shown in Fig. 5 could be partly explained by a possible labilization of phage RNA in the absence of coat protein synthesis during amino acid starvation. This would result in replenishing of nucleotide pools from phage RNA turnover and a consequent block in exogenous uptake in the absence of nucleotide pool expansion (16). To test this possibility, an experiment was performed with starvation only for histidine. This amino acid is absent from R23 coat protein, the synthesis of which will thus proceed in its absence (22). Starvation for only histidine, starting at 40 min after infection, almost completely inhibited incorporation of radioactive uracil into phage RNA, with a pattern very similar to that obtained with starvation for other amino acids (Fig. 6).

Determinations of guanosine tetraphosphate in the stringent strain Cp78 F^+ with and without phage infection and with amino acid starvation as described in Fig. 5 showed a rapid increase in ppGpp in both cases. The rise was, however, repeatedly found to be somewhat slower and to reach a slightly lower maximum level in the infected than in the uninfected bacteria. In one experiment the ppGpp concentration in uninfected Cp78 F^+ thus increased from 50 to 600 pmol per 10^8 cells during the first 5 min of amino acid starvation to reach a figure of 733 after about 20 min and then decreased to

FIG. 6. Incorporation of [^{14}C]uracil into RNA of R23-infected stringent and relaxed host strains during histidine starvation. Cells of Cp78 F^+ , Cp78 F^- , and Cp83 F^+ were grown and infected at $0.5 \times 10^9/\text{ml}$ in the low-phosphate mineral salts medium. Histidine was removed by filtration at 40 min after infection, and 3 min later [^{14}C]uracil ($0.1 \mu\text{Ci/ml}$, $2 \mu\text{g/ml}$) was added. RNA was determined in 0.1-ml samples as described in the text. Histidine was added back at 80 min after infection. Values along the ordinate denote counts per minute per 0.1-ml sample. Symbols: $\circ$, Cp78 F^+ ; Δ , Cp83 F^+ ; $\times$, Cp78 F^- .

717 at 40 min. In R23-infected Cp78 F⁺, ppGpp rose from 50 to 417 pmol per 10⁸ cells in 5 min and to 550 after 20 min, and finally reached a value of 617 after 40 min of amino acid starvation. The dramatic changes induced by amino acid starvation in the rates of RNA synthesis as measured in the described experiments could be due to the primary restriction of the uptake of uracil and adenine, respectively, into precursor nucleotide pools. To investigate this possibility, the relative specific activity of the UTP and ATP pools was determined under different labeling conditions. Cells were exposed to [³²P]phosphate for several generations and then labeled during short times with [³H]adenine or [³H]uracil. In Table 1 are shown the fractions of the total UTP and ATP pools labeled during 1-min pulses of the exogenous precursors, given at different times after amino acid removal from the stringent Cp78 F⁺, with and without phage infection. In an uninfected culture the entry of exogenous [³H]uracil into the intracellular UTP pool was reduced from 23% to about 1% during amino acid starvation. After readdition of amino acids the uptake rapidly rose several-fold, although the prestarvation level was approached only slowly. A similar pattern was seen in an infected culture where the uptake decreased somewhat more slowly from 20.5% to about 1.5% under amino acid starvation. When the above experiments were repeated with [³H]adenine as a precursor, in an uninfected culture the adenine uptake into the ATP pool decreased from 50% to about 20% during starvation and then rapidly returned to the prestarvation value at readdition of amino acids (Table 1). In the infected culture the [³H]adenine uptake was less restricted during starvation. It decreased from 63% to about 35%. Table 1 thus demonstrates that the incorporation of both uracil and adenine into their respective nucleotide pools is restricted during amino acid starvation, but slightly less so in R23-infected than in uninfected host bacteria, and much less so for adenine than for uracil. The pool labeling fractions of Table 1 could then be used to further test the dramatic changes in RNA synthesis observed in cumulative incorporation experiments like those described in Fig. 5 and 6. To do this, rates of RNA synthesis were measured under different conditions by short-term incorporations of radioactive precursor. The effect of amino acid starvation on 75-s incorporations of [³H]adenine into RNA of the stringent strain Cp78 F⁺ is shown in Fig. 7A. During starvation, the labeling rapidly decreased with time after amino acid removal. The same general pattern

TABLE 1. Uptake of [³H]uracil and [³H]adenine into the UTP and ATP, respectively, of Cp78 F⁺ during amino acid starvation and with and without phage infection^a

Cells	[³ H]uracil		[³ H]adenine	
	Min ^b	(pmol of [³ H]UTP)/(pmol of [³² P]UTP) × 100 ^c	Min	(pmol of [³ H]ATP)/(pmol of [³² P]ATP) × 100
Uninfected	30	23	30	50
	43	1.9	43	38
	58	0.5	58	17
	76	0.9	76	19.5
	88	7.7	88	54
	110	17.7	110	53
Infected	30	20.5	30	63
	43	5.4	43	45
	58	1.5	58	33
	76	1.2	76	36
	88	7.7	88	42
	110	13.6	110	49

^a Cells were grown and infected in the presence of [³²P]-phosphate as described in the text.

^b Labeling with [³H]uracil or [³H]adenine took place during 1-min periods starting at the indicated times. Amino acids were removed at 40 min after infection (or start of experiment) and added back at 80 min.

^c Molar amounts of ³²P- and ³H-labeled nucleotides were calculated from samples of known concentrations added to thin-layer plates as described in the text.

was seen after R23 phage infection (Fig. 7B), although in this case the decrease was less pronounced. In the relaxed strain Cp83 F⁺, infected with R23, the short-term incorporations were completely unaffected by amino acids starvation (Fig. 7C).

The data of Fig. 7 were combined with the specific activities, presented in Table 1 for the ATP pool at corresponding times, to give the relative rates of RNA synthesis. The resulting, corrected rates of RNA synthesis are shown in Fig. 8 for Cp78 F⁺ during amino acid starvation and with and without R23 phage infection. The infected and noninfected cultures, respectively, of the stringent strain showed a very similar, stringent response to amino acid starvation, which indicates that the phage-specific RNA-synthesis was also under the control of the *rel* gene of the host.

DISCUSSION

The aim of these experiments was to study the effect of the *rel* gene on an RNA synthesis

FIG. 7. Rates of RNA synthesis during amino acid starvation of R23-infected stringent and relaxed host bacteria. At times indicated on each curve, 500- μ l samples were transferred to tubes containing [3 H]adenine and incorporation into alkali-labile product was measured as described in the text. In each experiment, amino acids were removed at 40 min after infection (or start of experiment) and added back at 80 min. Values along the ordinate denote counts per minute incorporated per 50- μ l sample. (A) Uninfected Cp78 F $^+$; (B) R23-infected Cp78 F $^+$; (C) R23-infected Cp83 F $^+$. Symbols: (●) [3 H] added 30 min after infection; (×) 43 min after infection; (■) 58 min after infection; (▲) 77 min after infection; (○) 88 min after infection; and (■) 110 min after infection.

that is independent of the DNA-directed RNA polymerase of *E. coli*. The RNA-dependent RNA synthesis of phage R23 was chosen for examination because of its remarkable property of completely dominating host RNA formation after infection.

Earlier studies on the stringent regulation of phage RNA synthesis yielded conflicting results. Thus the replication of phage f2 was observed to be dependent on the presence of amino acids in a system where host RNA synthesis was inhibited by rifampin (9). In contrast, the formation of RNA by the serologically related phage R17 in a similar rifampicin-inhibited system was found not to be governed by the *rel* gene of the host (23). Finally, determination of infectious Q β RNA formation by a spheroplast assay indicated independence of amino acid starvation in both stringent and relaxed hosts (20).

In the present work the domination of R23 RNA synthesis in the infected cells obviated the use of rifampin, and the careful correction for variations in precursor pool uptake was attempted.

The reported inhibition of host RNA synthesis by R23 (22), which is important for the approach used, was checked by electrophoretic analysis. The phage was clearly seen to interfere with the ribosomal labeling pattern. The labeling of both the 16S and the 23S RNA peaks was abolished at 40 min after phage infection. Very

little radioactivity, and only in a diffuse pattern, was incorporated in the area corresponding to these peaks when label was supplied between 40 and 60 min after infection.

At a time period after infection when phage interference with rRNA synthesis was well established and phage RNA synthesis ought to have dominated, the cumulative incorporation of both adenine and uracil was severely restricted by amino acid starvation of the stringent strain, whereas the relaxed strain showed unaffected incorporation. This inhibition of incorporation was not due to labilization of phage RNA in the absence of coat protein formation, since the pattern was very similar during late starvation for only histidine, which is not part of the coat protein. Furthermore, the unaffected incorporation during starvation of relaxed cells argues against such an interpretation.

Earlier reports demonstrated that the uptake of uracil into the UTP pool of stringent bacteria was severely inhibited during amino acid starvation (24), and also that the uptake of adenine into ATP was much less affected under similar conditions (13). In the present investigation adenine was used in short-term incorporations to examine further the dramatic effect of starvation on the RNA synthesis of R23-infected bacteria. The inhibition of adenine uptake into the ATP pool was corrected for by determining the specific activities of the ATP to obtain true rates of RNA synthesis at different times during

FIG. 8. Relative rates of RNA synthesis in Cp78 F⁺ with and without R23 phage infection. The corrected rates of RNA synthesis were obtained by dividing the radioactivity incorporated from [³H]adenine into RNA in 1 min (cf. Fig. 7A and B) by the specific activity of the ATP pool at each time of sampling (cf. Table 1). Amino acids were removed at 40 min after infection (or start of experiment) and added back at 80 min. Values below bars denote time from phage infection (0 min). Symbols: Filled bars, infected Cp78 F⁺; unfilled bars, uninfected Cp78 F⁺.

amino acid starvation. These rates in phage-infected bacteria under starvation decreased similarly to those of uninfected bacteria, indicating a stringent regulation also of phage-specific RNA synthesis. This is in concurrence with the formation of ppGpp in stringent R23-infected bacteria during starvation and the recent finding that the initiation of Q β RNA synthesis in vitro is inhibited by ppGpp (12).

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