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Growth of Ribonucleic Acid Bacteriophage f2 in a Conditional Putrescine Auxotroph of *Escherichia coli*: Evidence for a Polyamine Role in Translation

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The ribonucleic acid (RNA) bacteriophage, f2, grows poorly in a conditional putrescine auxotroph during polyamine starvation. The addition of putrescine simultaneously with f2 enhances phage growth, shortens the latent period, and increases the burst size. The stimulation of f2 growth is reflected in higher rates of phage RNA and protein syntheses as measured by radioactive labeling of infected cells in the presence of rifampin. Putrescine does not affect f2 adsorption or the penetration of its RNA. Rather, in vitro assays demonstrate that in putrescine-supplemented cells more molecules of f2 replicase are made per incoming parental RNA than in polyamine-starved cultures. The ability of polyamines to stimulate the translation of a preformed messenger suggests a physiological role for these organic cations in normal protein synthesis.

The polyamines putrescine and spermidine, found as natural products in most organisms, have been shown to be necessary for rapid growth in *Escherichia coli* through the use of a mutant conditionally incapable of synthesizing the initial precursor, putrescine (7, 11, 16a, 24). Earlier (24), we demonstrated that the addition of putrescine (or spermidine) to cells partially depleted of their polyamines initiated a definite sequence of macromolecular events, the first detectable effect being the stimulation of protein synthesis (24). After approximately 60 min, increases in stable ribonucleic acid (RNA) and deoxyribonucleic acid syntheses and an acceleration in the rate of cell division occurred. Those past experiments were not designed to measure changes in messenger RNA (mRNA) synthesis.

The immediate stimulation of protein synthesis suggests that polyamines are needed in a normal cell for mRNA synthesis and/or for the efficient translation of pre-existing messengers. To test the second possibility, it was decided to utilize the special properties of the male-specific RNA bacteriophage f2. This RNA phage (12, 20, 26) carries its own preformed messenger. After infection, the parental "plus" strand of 10^6 molecular weight, bearing information for only three genes, is partially translated into the phage-specific subunit of the f2 replicase (2, 4, 9, 14). Synthesis of double-stranded RNA and progeny plus strand RNA, and the translation of the other two genes for coat and maturation proteins, follow in a definite order (12, 20, 26). Since f2 inserts a preformed message whose immediate translation product, the f2 replicase, can be assayed (2, 3, 9, 14), it was felt that this system provided a means of uncoupling translation from host transcription and offered an opportunity to observe, in vivo, any direct effect of polyamines on translation.

MATERIALS AND METHODS

Bacterial strains. The male-specific, RNA bacteriophage, f2, was kindly provided by Peter Model. *E. coli* K-12 strain C600 (K19) (5, 23), also provided by P. Model, was used to prepare large phage stocks and served as the indicator strain for f2. The conditional putrescine auxotroph, *E. coli* K-12 strain MA-135, (F^-, *argE*, *his*, *pro*, *trp*, *thi*, *speB*, *canR*) whose microbiological properties have been described extensively elsewhere (11, 24), was converted into a suitable male host for f2 by bacterial mating with the male donor strain KLF 15/JC 1553 (F' *his*$^+$*/his*, *leu*, *met*, *arg*, *lacY*, *str*, *recA*) provided by Werner Maas. Transfer of the male genotype was indicated by loss of the histidine requirement in the female recipient and by the ability to grow f2. The conditional putrescine requirement was tested as previously described (11, 24) on arginine-containing plates. A suitable male, conditional putrescine auxotroph, designated strain ED-5 (F', *his*$^+$*/his*, *argE*, *pro*, *trp*, *thi*, *canR*, *speB*), was used in the following experiments.

Biochemicals. The polyamines, putrescine dihydrochloride and spermidine trihydrochloride, were

purchased from Calbiochem. Rifampin was supplied by the Dow Chemical Co. Ribonuclease-free deoxyribonuclease I was obtained from Worthington Biochemical Corp., whereas pancreatic ribonuclease was purchased from both Worthington and Sigma Chemical Co. The sodium salts of adenosine 5'-triphosphate (ATP), uridine 5'-triphosphate (UTP), cytidine 5'-triphosphate (CTP), and tetralithium guanosine 5'-triphosphate (GTP) were all purchased from Schwarz/Mann, neutralized to pH 7.0, and stored frozen at −20 C before use. The tricyclohexylammonium salt of phosphoenolpyruvate (converted to the potassium salt, neutralized to pH 7.0, and stored frozen at −20 C before use) and phosphoenolpyruvate kinase were the products of Boehringer Mannheim Corp. [5-^{3}H]uracil (500 μCi/ml, 7,800 μCi/μmol), L-[^{14}C]leucine uniformly labeled (50 μCi/ml, 312 μCi/μmol), L-[^{14}C]histidine uniformly labeled (50 μCi/ml, 312 μCi/μmol), and [^{3}H]GTP (1,000 μCi/ml, 1,380 μCi/μmol) were all purchased from Schwarz/Mann. The f2 antiserum used in the adsorption experiments was the generous gift of Peter Model.

Bacterial media. Strain ED-5 was grown in either minimal medium or enriched, synthetic medium. The minimal medium (24) contained the Davis-Mingioli salts medium (6) plus 0.25% glucose, proline (100 μg/ml), tryptophan (100 μg/ml), and thiamine (2 μg/ml). When either ornithine (100 μg/ml), arginine (200 μg/ml), or arginine plus putrescine (100 μg/ml) were added, the medium was designated MMO, MMA, or MMAP, respectively. The enriched, synthetic medium, AF′, was a modification of the previously described AF medium (11). It contained the low phosphate, tris(hydroxymethyl)aminomethane (Tris)-maleate buffered salts mixture (17), plus 0.5% glucose, all the common amino acids (except for histidine, leucine, lysine, arginine, and ornithine), adenosine, and vitamins (including thiamine). Since f2 penetration requires Ca^{2+} (19), a low-phosphate medium was necessary to prevent precipitation of calcium phosphate. The precise concentrations of all of the above components can be found in earlier references (11, 17). For the radioactive labeling experiments, uracil, leucine, and histidine were added as called for below. In general, when they were not used as labels, these compounds were added as supplements. As with the minimal media, when ornithine, arginine, or arginine plus putrescine were added, the enriched, synthetic medium was designated AF′, AF′A, or AF′AP, respectively. For details concerning the high-phosphate media AFO and AFA a previous report should be consulted (11).

Tryptone broth (15) was used for the growth of strain K19.

Polyamine starvation procedure. Polyamine-starved cells were prepared by the following procedure (24). Strain ED-5 was grown overnight at 37 C with aeration in MMO medium. The culture was then refrigerated for a minimum of 4 h at 4 C. A 1:20 inoculum was added to MMA medium and allowed to grow with aeration at 37 C overnight. The following morning the cells were centrifuged and resuspended (5- to 10-fold diluted) in AF′A medium or another suitable medium.

Radioactive labeling of phage-specific RNA and protein. A polyamine-starved culture of strain ED-5, prepared as outlined above, was centrifuged and resuspended (5- to 10-fold diluted) in AF′A medium. After growth at 37 C with aeration for 3 h, [5-^{3}H]uracil (0.25 μCi per 10 μg per ml) and L-[^{14}C]leucine (0.30 μCi per 10 μg per ml) were added to the culture. One hour later the culture was divided into three portions; all portions received f2 (multiplicity of infection [MOI] ~30) and $CaCl_2$ (2.5 mM). One minute later putrescine (100 μg/ml) was added to one culture (early putrescine addition). At 10 to 12 min after infection, rifampin (25 μg/ml) was added to all cultures. At 16 min postinfection, putrescine was added to a second culture (late putrescine addition). Samples of 0.5 ml were removed from all cultures at the indicated intervals and added to 0.5 ml of cold trichloroacetic acid (10%). The precipitates were collected on GF/C filters, washed with 5% trichloroacetic acid, dried, and counted in 2,5-diphenyloxazole-1,4-bis-2-(5-phenyloxazolyl)benzene toluene with a Nuclear-Chicago Mark I liquid scintillation counter.

Preparation of f2 replicase containing infected cell extracts and the f2 replicase assay. The following procedure for the preparation of infected cell extracts and the f2 replicase assay were derived with minor modifications from previously published methods (2, 3, 9, 14).

Polyamine-starved or putrescine-supplemented cultures of strain ED-5 cells (10^8 to 2×10^8/ml), at various times after infection with f2 (MOI~5), were poured over frozen, crushed saline and collected by centrifugation at 4 C. Each cell pellet (stored at −20 C) was ground with twice its weight of alumina at 4 C for a few min. A volume of starting buffer (50 mM Tris-chloride buffer, pH 7.5, 5 mM magnesium acetate, 1 mM ethylenediaminetetraacetic acid, 0.1 mM dithiothreitol, and 25% [vol/vol] glycerol) equal to eight times the cell weight was added to each extract and the mixture was centrifuged twice at 30,000 × *g* for 10 min at 4 C. The supernatant, containing f2 replicase activity, was stored at −70 C.

The f2 replicase assay was carried out in a total volume of 0.2 ml and contained 0.125 M potassium phosphate buffer, pH 7.0, 0.5 mM dithiothreitol, 10 mM magnesium acetate, 0.25 mM each of ATP, CTP, and UTP, ribonuclease-free deoxyribonuclease I (25 μg/ml), rifampin (5 μg/ml), 10% (vol/vol) glycerol, 5 mM phosphoenolpyruvate, potassium salt, phosphoenolpyruvate kinase (2 μg/ml), 0.1 mM [^{3}H]GTP (2 μCi/assay, 100 μCi/μmol, or 44 counts per min per pmol of GMP incorporated), and infected cell extract in varying amounts. The reaction mixture was incubated at 25 C for 10 min and terminated by the addition of 3 ml of cold trichloroacetic acid (5%) containing 0.02 M sodium pyrophosphate. The precipitates were collected on GF/C filters, washed repeatedly with 5% trichloroacetic acid which contained 0.02 M sodium pyrophosphate, dried, and counted as above. As a suitable background the trichloroacetic acid-precipitable activity found in uninfected cell extracts was subtracted from infected cell activity at each protein concentration. The protein concentrations in infected and uninfected extracts were determined by the method of Lowry et al. (16).

RESULTS

One-step growth curves. For the purpose of our experiments, the conditional putrescine auxotroph, MA-135, was converted by conjugation to a male strain, ED-5. Strain ED-5, which like strain MA-135 (11, 24) cannot synthesize putrescine when arginine is present in the external medium and ornithine is absent. It grows with a generation time of 45 min in tryptone broth and 90 min in the enriched synthetic medium, AFO. After overnight starvation for polyamines, a generation time of 200 to 300 min is obtained in the starvation medium, AFA. The procedure used here to starve the cells of their endogenous pools of polyamines causes the intracellular putrescine to fall to below detection levels, whereas spermidine is present at one-third to one-half of the value observed in putrescine-supplemented cultures (11).

When f2 is grown in strain ED-5 under the different culture conditions cited above, the latent period of phage development and the final burst size reflect the cellular metabolic rate at the time of infection. The latent period in tryptone broth is 30 min, in AF'O medium 50 to 60 min, and for polyamine-starved cells in AF'A medium it is 80 to 90 min. Similarly, the burst size is highest in tryptone broth and for nonstarved cells in AF'O medium and lowest for polyamine-starved cells in AF'A medium.

To investigate the effects of polyamine starvation and supplementation on f2 growth, polyamine-starved cells were grown in AF'A medium and infected with phage. Either shortly before or simultaneously with phage infection, putrescine was added to the cultures (Fig. 1). It can be seen (Fig. 1) that putrescine increases the initial burst size (at 180 min) and shortens the latent period. Addition of putrescine 20 min prior to infection raises the burst size to a level near that of nonstarved, infected cells grown in an AF'O medium (unpublished data). At times very late after infection (300 min), the phage yield of the starved culture begins to approach that of the culture supplemented with putrescine at the time of infection.

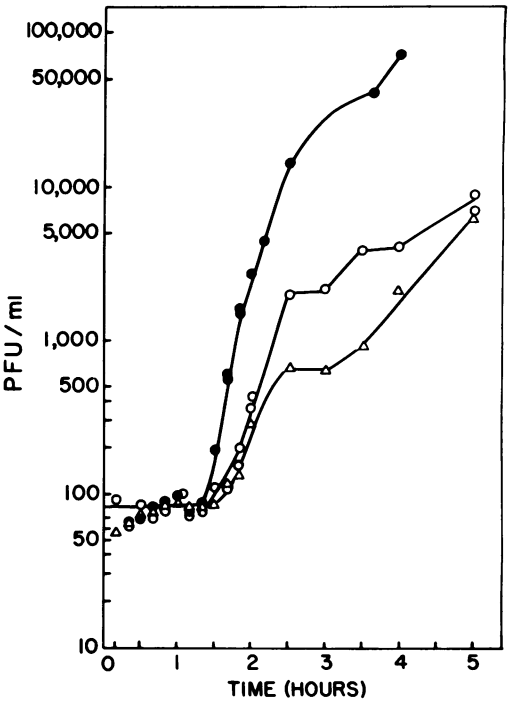

FIG. 1. *Effect of polyamine starvation on the one-step growth of f2 in strain ED-5. Polyamine-starved cells of strain ED-5 (see Materials and Methods) were resuspended in AF'A medium (supplemented with histidine and leucine) and grown at 37 C for ~3 h to a cell density of 0.6 × 10⁸/ml. At −20 min the culture was divided into three portions and one portion received putrescine. At −5 min putrescine was added to a second portion and all three then received NaCN (2 mM), $CaCl_2$ (2.5 mM), and f2 (MOI ~ 0.12). At time zero the cultures were diluted 10^{-4} and titrated by standard procedures (1, 15) without chloroform for extracellular phage released at the indicated times. The data presented as total PFU/ml includes both adsorbed and unadsorbed phage. In this experiment about one-third of the initial PFU represents adsorbed phage as determined by chloroform treatment. Strain K19 served as the indicator bacterial strain. Incubation of all tryptone broth-agar plates (15) was at 37 C overnight. Symbols: Δ, no putrescine; O, putrescine added with f2; ●, putrescine added at −20 min.*

Phage-specific RNA and protein syntheses. Rifampin is a well-known inhibitor of *E. coli* RNA polymerase (13, 22). When added to an exponentially growing culture of strain ED-5, rifampin (25 μg/ml) immediately stops RNA synthesis, as measured by radioactive incorporation, and within 5 min protein synthesis also ceases (Fig. 2). Since f2 does not shut down host RNA and protein syntheses, it is necessary to add rifampin to infected cultures in order to measure radioactively labeled phage RNA and protein (10).

Figures 3 and 4 summarize the results of a typical double-label experiment in which phage protein was labeled with [^{14}C]leucine and phage RNA was labeled with [^{3}H]uracil. In this experiment a polyamine-starved culture growing in an AF'A medium was divided into three parts. At zero time all cultures received f2 and $CaCl_2$. The first culture also received putrescine

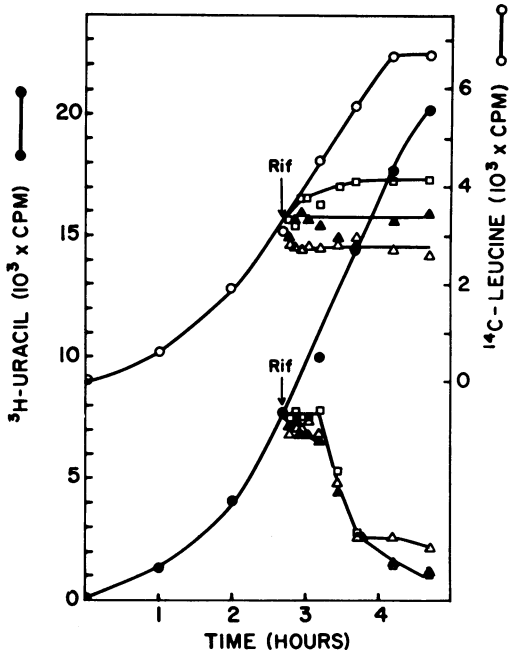

FIG. 2. *Inhibition of host RNA synthesis by rifampin in exponentially growing cells. An overnight culture of strain ED-5 in MMO medium was centrifuged and resuspended (10-fold diluted) at time zero in AF'O medium containing* L-[^{14}C]*leucine (0.05 μCi per 5 μg per ml) and [5-*^3H]*uracil (0.25 μCi per 10 μg per ml). At the indicated times, 0.5-ml samples were removed and mixed with 0.5 ml of cold 10% trichloroacetic acid. At 2 h and 40 min, the culture was divided into four parts and rifampin was added to three of them at the concentrations of 10 μg/ml (□), 25 μg/ml (▲), and 50 μg/ml (△). The fourth portion (○, ●) remained rifampin free. The samples were treated and counted, and the* ^3H *and* ^{14}C *counts were distinguished as described in Materials and Methods.*

1 min after infection (early putrescine). At 11 min postinfection, rifampin (25 μg/ml) was administered to all the cultures. Finally, at 16 min, putrescine was added to the second culture (late putrescine). The third culture did not receive any putrescine. Both figures clearly show that restoration of the cellular polyamine level causes significant increases in the rates of phage RNA and protein syntheses. The time of putrescine addition either before or after rifampin addition does not seem to affect the labeling curves (the difference seen in the RNA curve was not confirmed by other experiments). The stimulatory effects of polyamines must, therefore, occur independently of the host transcriptional system. Also, it should be noted that in the putrescine-supplemented culture there is a distinct shut-off of both RNA and protein syntheses which is absent in the polyamine-starved culture during the course of the experiment.

Effect of putrescine on f2 adsorption and penetration. Although the results obtained thus far suggest that polyamines can exert at least some of their stimulatory effects on f2 growth independently of host RNA transcription, the exact cause of these effects is still unclear. They might either act at some step in the intracellular development of the phage, or simply assist in f2 adsorption and penetration.

Table 1 shows that putrescine does not affect the adsorption of f2 to bacterial cells. In this

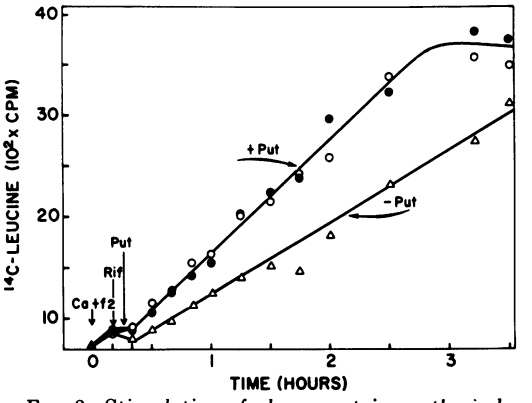

FIG. 3. *Stimulation of phage protein synthesis by polyamines. The exact experimental protocol is given in detail in Materials and Methods. Rifampin (25 μg/ml) was added at 11 min postinfection. Symbols: △, no putrescine added; ○, putrescine added 1 min after phage; ●, putrescine added 16 min after phage.*

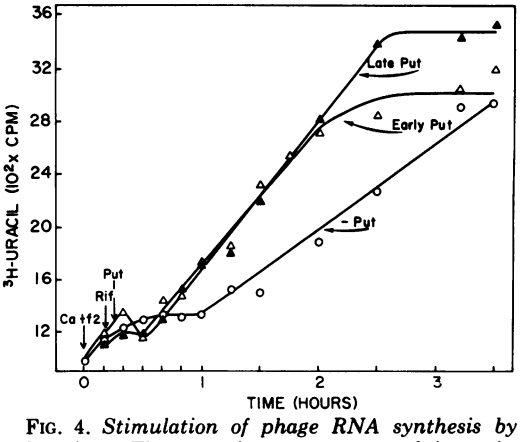

FIG. 4. *Stimulation of phage RNA synthesis by polyamines. These results were computed from the double-label experiment described in Fig. 3. Symbols: ○, no putrescine added; △, putrescine added 1 min after phage (Early Put); ▲, putrescine added 16 min after phage (Late Put).*

TABLE 1. *Effect of putrescine and spermidine on f2 adsorption*[a]

Determination	Infection media	
	AF'A (polyamine-starved)	AF'AP (polyamine-supplemented)
Bacterial cell count[b]/ml	6.8×10^6	6.8×10^6
f2 Input (PFU/ml)	3.3×10^7	3.3×10^7
Total PFU/ml	2.62×10^7	2.33×10^7
Infectious centers/ml	1.74×10^6	1.85×10^6
Chloroform-treated supernatant (PFU/ml)	1.7×10^5	2.7×10^5
Corrected infectious centers/ml	1.57×10^6	1.58×10^6
Bacteria infected (%)	23.1	23.25
Input f2 producing infectious centers (%)	4.76	4.79

[a] A culture of polyamine-starved strain ED-5 cells, after growth in AF'A medium at 37 C to a density of 0.5×10^8 to 1.0×10^8 ml, was divided, and putrescine (100 μg/ml) was added to one portion. Immediately, $CaCl_2$ (2.5 mM) and f2 (MOI ~ 3 to 5) were added, and phage adsorption was permitted for 10 min at 37 C. The infected cultures were then diluted 1:10 with the Tris-maleate buffered salts medium (17) containing 2.5 mM $CaCl_2$, and the total plaque-forming units (PFU) of adsorbed and unadsorbed phage ("total PFU") of the diluted cultures was measured. The diluted cultures then received f2-specific antiserum and were kept at room temperature for 15 min to remove unadsorbed phage. The cultures were then titrated to determine the amount of adsorbed f2 ("infectious centers"). Part of the remaining diluted cultures were treated with $CHCl_3$ at 37 C for 30 min and centrifuged, and the supernatant solutions were titrated to measure any unadsorbed phage which escaped antiserum neutralization ("chloroform-treated supernatant"). This number was subtracted from the value obtained for "infectious centers" to yield a better estimate of the adsorbed phage ("corrected infectious centers"). The "bacteria infected (%)" and the "input f2 producing infectious centers (%)" were computed by dividing the values for the "corrected infectious centers" by the "bacterial cell count" and the "f2 input."

[b] The "bacterial cell count" was determined for the 1:10 diluted cultures.

experiment a polyamine-starved culture was infected with f2 (MOI~5) in the presence and absence of putrescine. Ten minutes later the cultures were diluted 10-fold and titrated for f2 plaque-forming units, and sufficient f2 antiserum was added to remove all unadsorbed phage. After an additional 15 min the cultures were again titrated and treated with chloroform, and the chloroform-treated supernatant was re-titrated. The corrected values for the number of infectious centers were equal for both cultures. Putrescine does not increase the fraction of bacteria susceptible to f2 adsorption.

Similarly, the addition of putrescine at the time of infection does not facilitate the penetration of f2 RNA into the host cell. It is known that ribonuclease and rifampin added prior to f2 infection inhibit f2 plaque formation almost completely (8, 10, 19, 25; unpublished data). Addition of rifampin after infection causes less inhibition of plaque formation, the degree of inhibition being dependent on the time of addition of the drug after infection (8; unpublished results).

The effect of ribonuclease, added prior to f2, on phage-specific RNA labeling is illustrated in Fig. 5. In polyamine-starved cells, ribonuclease greatly reduces phage RNA synthesis for nearly 2 h, although RNA is rapidly made later. Putrescine supplementation causes an increase in the labeling rate and this is also true in the cultures devoid of exogenous ribonuclease (Fig. 4) and probably reflects a stimulation of intracellular events. On the other hand, if ribonuclease is added 12 min after f2 infection there is no effect on phage RNA labeling either in the absence or presence of putrescine (Fig. 6). Putrescine added before (at 1 min) or after ribonuclease (at 16 min) elicits no change in the labeling pattern. In polyamine-starved cultures,

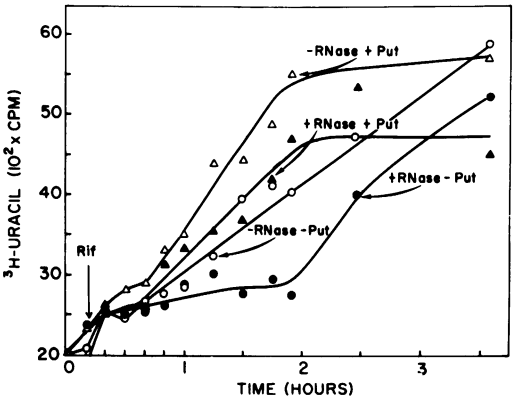

FIG. 5. *Inhibition of phage RNA synthesis by ribonuclease added prior to infection. The experimental protocol followed closely the procedure described in Materials and Methods for radioactive labeling of phage RNA with the major exception that ribonuclease (20 μg/ml) was added to all four cultures 10 min before infection with f2 (time zero). Putrescine and rifampin were added at 1 and 12 min after infection, respectively. Symbols: ○, no putrescine added, no ribonuclease added; ●, no putrescine, but with ribonuclease addition; △, putrescine added, but no ribonuclease; ▲, putrescine added, ribonuclease added.*

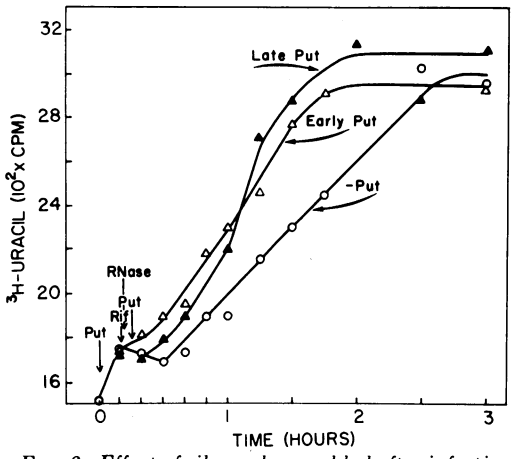

FIG. 6. *Effect of ribonuclease added after infection on phage RNA synthesis. The experimental procedure is identical to the experiment of Fig. 3 and 4 with the exception that ribonuclease (20 μg/ml) was added to all three cultures 12 min after f2 infection. Symbols: O, no putrescine; Δ, putrescine added 1 min after phage (Early Put); ▲, putrescine added 16 min after phage (Late Put).*

f2 adsorption and penetration are completed by 12 min after infection. Therefore, in the experiments depicted in Fig. 3 and 4, putrescine addition at 16 min could not have increased the labeling rates by enhancing the efficiency of f2 penetration. Since early and late additions yield nearly identical labeling patterns, it is also unlikely that early addition of putrescine affects f2 penetration. The same conclusions can be made from similar experiments in which rifampin, instead of ribonuclease, was added before infection.

Stimulation of f2 replicase synthesis by putrescine. The first known event following the binding of the incoming parental f2 RNA to host ribosomes is the translation of the replicase gene into the phage-specified subunit of the f2 replicase (2, 4, 9, 14). All of the replicase molecules produced in infected cells come from this initial, host transcription-independent translation of the parental RNA (14, 18). Furthermore, it has been shown that the f2 replicase assay, in which contributions from host RNA synthesizing systems are effectively prevented, gives a good approximation of the actual number of replicase molecules (10, 12, 21). By direct in vitro assay of the f2 replicase synthesized after infection, it is possible to determine whether polyamines directly affected this initial, translational event.

The addition of putrescine does augment the synthesis of f2 replicase in infected cells (Fig. 7). The first observable activity appears 15 min after infection and exhibits a slow, linear increase in polyamine-starved cells. Putrescine supplementation at the time of infection does not appreciably alter the time of initial appearance of enzyme, but does increase its rate of synthesis: the maximum difference occurs shortly after 20 min. Eventually, this rapid replicase synthesis in supplemented cells is terminated and replaced by a slower accumulation of enzyme. The replicase accumulation curves for both starved and supplemented cultures bear a close resemblance to the labeling curves of Fig. 3, 4, and 6, with the exception that shut-off occurs at an earlier time in Fig. 7.

The higher specific activity of extracts from putrescine-supplemented cultures can be demonstrated over a wide range of protein concentrations (Fig. 8), and is presumably due to an actual increase in the number of enzyme molecules. The maximum difference between supplemented and starved cultures is observed 20 min after infection. The kinetics of the polymerization reaction using 20-min extracts (Fig. 9) again reveals the considerably higher specific activity of the supplemented culture. Since polyamines added to an in vitro assay do not stimulate f2 replicase activity (Table 2), the possibility that polyamines simply enhance the activity of the enzyme without an actual in-

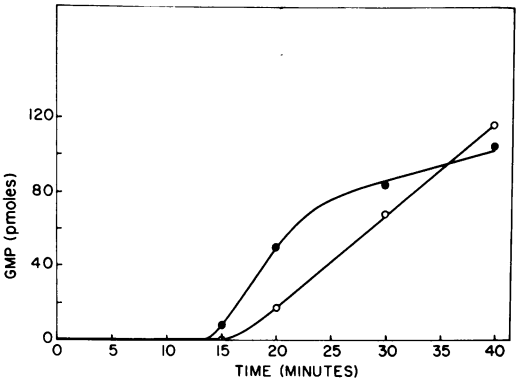

FIG. 7. *Effect of putrescine supplementation on the rate of appearance of f2 replicase activity in polyamine-starved, infected cells. The details for the growth and harvesting of infected cells, the preparation of infected cell extracts containing f2 replicase activity, and the f2 replicase assay are described in Materials and Methods. Putrescine was added to one culture at the time of infection (time zero). Rifampin was not added to the cells prior to nor during infection. Symbols: O, replicase activity in the polyamine-starved culture; ●, replicase activity in the putrescine-supplemented culture.*

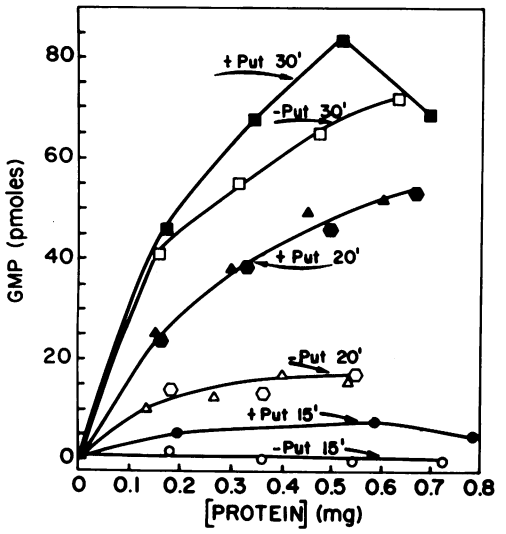

FIG. 8. *Effect of increasing protein concentration on f2 replicase activity from polyamine-starved and putrescine-supplemented infected cells. The details for infected cell extract preparation and the replicase assay appear in Materials and Methods. The abscissa indicates the amount of protein added per assay. The final assay volume was 0.20 ml in each case. Infected, polyamine-starved and putrescine-supplemented (at time of infection) cells were harvested at 15, 20, and 30 min after infection. The 20-min data show the results of two, separate, infected cell preparations for both starved and supplemented extracts. Symbols: ○, no putrescine, harvested at 15 min; ●, putrescine added, harvested at 15 min; △, ◇, no putrescine, harvested at 20 min; ▲, ◆, putrescine added, harvested at 20 min; □, no putrescine, harvested at 30 min, ■, putrescine added, harvested at 30 min.*

crease in the number of replicase molecules is ruled out.

DISCUSSION

We have previously suggested that polyamines in *E. coli* are involved in some aspect of protein synthesis (24). Because of the close association between transcription and translation in bacteria, it was not known whether polyamines influence transcription of mRNA, its translation, or both events. The RNA bacteriophage f2 was, therefore, used in the above experiments to dissociate host transcription from translation and to determine the effect, if any, of polyamines on the latter event.

The presence of polyamines enhances the growth of f2 (Fig. 1). This is seen in a shortened latent period and in an increase in the size of the initial burst (at 180 min). The labeling experiments confirm this conclusion; polyamines stimulate phage-specific protein and RNA labeling (Fig. 3 and 4), and the labeling curves obtained strongly resemble the one-step growth curves of Fig. 1. The high backgrounds of RNA and protein syntheses found in polyamine-starved cells may be due to residual levels of spermidine still present in these cells. Significantly, this stimulation by polyamines can occur in the absence of host RNA transcription, demonstrating the growth-stimulating ability of polyamines in a system in which transcription and translation have been uncoupled.

Also seen in these experiments is the influence of polyamines on the cessation of phage growth. Phage RNA and protein syntheses are clearly terminated by 150 to 180 min in the putrescine-supplemented cultures, whereas the polyamine-starved cells continue to synthesize

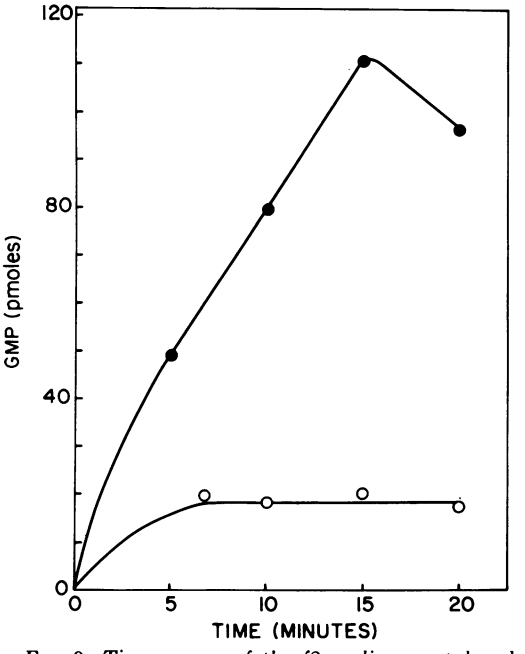

FIG. 9. *Time course of the f2 replicase-catalyzed reaction from polyamine-starved and putrescine-supplemented infected cell extracts. The preparation of infected cell extracts and the replicase assay were carried out as before. Putrescine was added to one culture at the time of infection. In both cases the infected cells were harvested 20 min after infection. The actual amounts of protein used in each assay (0.20 ml) were 551 μg and 501 μg for starved and supplemented extracts, respectively. The results on the ordinate have been normalized to 1 mg of protein in each case. Individual assays in a series were terminated at the indicated times and processed for counting as described earlier (Materials and Methods). Symbols: ○, activity from polyamine-starved, infected cells; ●, activity from putrescine-supplemented, infected cells.*

phage products at a slow rate. This regulatory phenomenon, also seen in normal infection (12, 18), has been attributed to the gradual accumulation of coat protein molecules which inhibit f2 translation. The failure to observe this regulation in polyamine-starved cells during the course of the experiment suggests that coat protein molecules may not have accumulated in sufficient amounts.

Since Ca^{2+} or other divalent metal ions are required for f2 penetration (19), it was necessary to explore the possibility that putrescine assisted in either adsorption or penetration. This possibility was clearly ruled out by experiments in which rifampin and ribonuclease were added prior to infection. Added before penetration, these agents inhibit phage RNA synthesis (Fig. 5 and reference 8); when added 12 min after f2 addition, however, they have no apparent effect on phage RNA labeling (Fig. 4 and 6). The process of penetration must be complete by 12 min in polyamine-starved cells, yet putrescine added at 16 min exerts effects on phage RNA and protein labeling identical to those obtained by early addition (Fig. 3 and 4).

The mechanisms by which ribonuclease and rifampin inhibit f2 growth are only incompletely understood. Ribonuclease presumably cleaves f2 RNA after exposure of this molecule prior to penetration (19, 25). Once nicked, the single-stranded parental strand cannot produce viable phage progeny. However, penetration and translation of this message does occur, since phage protein labeling is not affected by ribonuclease treatment prior to infection (unpublished results). Although nothing is known about the quality of these proteins made from nicked messengers, the sudden increase of phage RNA synthesis in ribonuclease-treated, polyamine-starved cells (Fig. 5) is perhaps due to a slow accumulation of active replicase molecules.

The results of the f2 replicase in vitro assays support the contention that polyamines can directly stimulate the translation of a preformed message. The kinetics of replicase appearance (Fig. 7) after infection of putrescine-supplemented cells resembles, in general, previously published reports (2, 14). It has been shown (10, 12, 21) by polyacrylamide gel electrophoresis of phage proteins from rifampin or actinomycin-treated infected cells that this increase in replicase activity after infection corresponds to an increase in the formation of replicase molecules. Presumably, more replicase molecules are made per parental RNA in the presence of putrescine than in its absence. Whether this means an increase in the number of translational initiation events or more complete translations of the entire gene is not known. However, the alternative interpretation that polyamines enhance f2 replicase activity, without increasing the number of enzyme molecules, is unlikely for the above-mentioned reasons. It is also unlikely because of the inability of polyamines to stimulate replicase activity when added to the in vitro assay (Table 2). Consequently, it may be tentatively concluded from these experiments that polyamines participate directly in some step of mRNA translation.

TABLE 2. *Effect of putrescine and spermidine added in vitro on f2 replicase activity*[a]

Additions	Incorporation of GMP (pmol)	
	Extract from polyamine-starved infected cells	Extract from polyamine-supplemented infected cells
None	22.8	49.2
0.05 mM spermidine	18.35	45.4
0.5 mM spermidine	16.95	42
5 mM spermidine	14.38	41.1
None	23.95	49.2
0.2 mM putrescine	23.95	47.7
2 mM putrescine	28.65	46.2
20 mM putrescine	16.58	43.6

[a] Infected cell extracts were prepared and f2 replicase activity was assayed as described in Materials and Methods. Incubation was for 10 min at 25 C. The polyamine-starved extract assays contained 404 μg of protein per assay; the putrescine-supplemented extract assays had 453 μg of protein per assay. Both were harvested 20 min after phage infection. One picomole of GMP incorporated equals 44 counts/min.

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