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Host Macromolecular Synthesis in Bacteriophage-infected *Bacillus subtilis*

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INTRODUCTION

Interest in *Bacillus subtilis* bacteriophages developed soon after Spizizen's (24) discovery of deoxyribonucleic acid (DNA)-mediated transformation in this organism. The *B. subtilis* transformation system allows the study of various factors affecting the biological activity of transforming DNA and the infectivity of specific viral nucleic acids in a bacterium capable of growth in a simple synthetic medium (21). Nutritionally less exacting than other organisms in which transformation has been studied, density and radioactive identification of macromolecular components in *B. subtilis* can be carried out with relative ease.

Independent *B. subtilis* phage isolates have been studied by Romig and Brodetsky (2, 22), by Marmur and Greenspan (12), and more recently by Okubo et al. (16), Földes and Trautner (5), Reilly and Spizizen (20), and Green (6). In a large number of these viruses, thymine is replaced in the viral DNA by an unusual base. Uracil replaces thymine in the transducing phage PBS1 isolated by Takahashi (25), and 5-hydroxymethyluracil (HMU) is present in SP8 (8), ϕe (23), and SPO1 (16), and probably occurs in related viruses such as SP82 (6). Of additional interest is the fact that, in all bacteriophages containing HMU, each complementary strand of the DNA duplex can be resolved on the basis of its buoyant density in cesium chloride, probably because of a bias in the distribution of purine and pyrimidines in the DNA (11).

The purpose of this communication is to report studies on the synthesis of host-specific macromolecules in bacteria infected with phage 2C, a virulent, HMU-containing bacteriophage active on transformable strains of *B. subtilis*. Some of the properties of the virus have been described (18).

MATERIALS AND METHODS

Bacterial Strains

All strains prefixed by the number 168 were derived from the transformable strain *B. subtilis* 168, an indole-requiring mutant isolated by Burkholder and Giles (3). *B. subtilis* 168-2 requires indole and leucine for growth; *B. subtilis* 168-3 requires indole and proline. *B. subtilis* A26 is trytophan-independent but requires uracil. All strains were maintained on Difco Veal Infusion Agar Plates.

Bacteriophage

Phage 2C was propagated on *B. subtilis* 168-2 by the methods outlined by Adams (1). Lysates freed from debris by low-speed centrifugation were concentrated in a Spinco type 19 rotor (19,000 rev/min; 60 min; 2C), and the pellets were resuspended overnight in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-0.01 M $MgSO_4$. The concentrated virus preparation was centrifuged at 6,000 $\times$ g for 10 min in a Sorvall refrigerated centrifuge at 2 C to remove additional debris. The supernatant solution was stored at 4 C.

Infection of B. subtilis with Bacteriophage 2C

Strains of *B. subtilis* growing in Spizizen's minimal medium (24) supplemented with 0.02% casein hydrolysate, 0.01% yeast extract, and their nutritional requirements (50 μg/ml for tryptophan, leucine, or uracil) were infected with concentrated stocks of bacteriophage 2C when cultures reached approximately 7×10^7 colony formers/ml. In logarithmically growing cultures of *B. subtilis*, extensive chain formation results in an underestimate of the number of cells since many colony formers are produced by doublets and triplets. The multiplicity of infection was, therefore, calculated from the natural logarithm of the surviving fraction (1) at multiplicities lower than two phages per bacterium. Higher multiplicities were calculated on the basis of the surviving fraction obtained from lower multiplicities.

Assay of Biological Activity of Phage and Bacterial DNA

Bacterial DNA was obtained by the method of Marmur (11). Competent cells of *B. subtilis* 168-2 and 168-3 were obtained as described by Pène and Romig (19) by use of a modification of the procedure published by Spizizen (24). Contact with DNA was limited to 30 min at 37 C by addition of 1 μg of pancreatic deoxyribonuclease per ml (Worthington Biochemical Corp., Freehold, N.J.). Transformants were enumerated by plating on selective synthetic medium supplemented with 0.1% of the appropriate amino acid assay medium (Difco).

Induction and Assay of D-Sorbitol Dehydrogenase

D-Sorbitol dehydrogenase was induced in *B. subtilis* A26 in L broth (Difco tryptone, 10 g; Difco yeast extract, 10 g; NaCl, 5 g; $MnCl_2$, 10^{-5} M; water, 1 liter) during the early logarithmic growth phase by addition of 1% D-sorbitol (final concentration). At various time intervals 100-ml amounts of induced bacteria were chilled in an ethyl alcohol-dry ice mixture, harvested by centrifugation in the cold, washed once with cold 0.5% NaCl-0.5% KCl, and resuspended in 3 ml of 0.05 M Tris-chloride buffer (*p*H 7.2) containing 2 mg of lysozyme per ml (Calbiochem, Los Angeles, Calif.). The suspension was incubated for 15 min at 37 C. Nucleic acids were precipitated with a final concentration of 1% streptomycin sulfate. The supernatant solution after centrifugation of the material was assayed for sorbitol dehydrogenase as described below. Measurement of enzyme induction in phage-infected cells at various times during the latent period was carried out at a multiplicity of infection of five phages per bacterium. Bacteria were induced as described above for 10 min.

Each assay cuvette contained, in a total volume of 1.0 ml, 15 μmoles of nicotinamide adenine dinucleotide (NAD; Sigma Chemical Co., St. Louis, Mo.), 50 μmoles of D-sorbitol, 60 μmoles of Tris-chloride buffer (*p*H 9), and a suitable amount of crude extract. After addition of crude extract (0.05 to 0.1 ml), the reduction of NAD was measured in a Zeiss spectrophotometer (340 nm) at room temperature within the 60- to 120-sec interval following addition of the extract. One unit of enzyme is defined as that amount which results in an increase of 0.01 optical density unit per min at 340 nm. Endogenous NAD reduction controls were performed in all cases by omitting D-sorbitol from the reaction mixture. Without exception, all of these controls showed no increase in absorbancy at 340 nm, indicating that no measurable endogenous NAD reduction occurred in the absence of substrate under the conditions of the experiment. Protein content was estimated by the method of Lowry et al. (10).

Measurement of Host DNA Synthesis

Host DNA synthesis was measured by the incorporation of 8 c/mmole of ^{3}H-thymidine (Nuclear Chicago Corp., Des Plaines, Ill.) into trichloroacetic acid-precipitable material. Infected bacteria (3 ml) were pulsed in L broth with 50 μc of ^{3}H-thymidine for 3 min at various times during the latent period. Uptake of radioactivity was terminated by the addition of 3 ml of 20% trichloroacetic acid containing 2 mg of thymidine per ml, and the sample was chilled rapidly. Cells were collected by centrifugation and washed once with 5% trichloroacetic acid. The pellet was suspended in 5% trichloroacetic acid and passed through membrane filters, type HA (Millipore Corp., Bedford, Mass.). Filters were dried and counted in a toluene 2-(4-*t*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxdiazole scintillator (Butyl-PBD, Beckman Instruments, Fullerton, Calif.) in a Beckman liquid scintillation counter.

Isolation and Fractionation of Ribosomal Ribonucleic Acid (RNA)

To measure the rate of synthesis of ribosomal RNA in *B. subtilis*, 25 ml of cells growing in minimal medium was pulsed with 0.1 μc of ^{14}C-uridine for 2 min at 37 C. After 2 min of uptake, 5 μg of actinomycin D per ml and 200 μg of cold uridine per ml were added, and incubation was continued for 6 min. Samples were poured on physiological saline ice containing 0.01 M sodium azide, centrifuged at 12,000 × *g* for 10 min, and resuspended in 3 ml of 0.15 M NaCl-0.01 M

NaN_3-0.01 M Tris (*p*H 7.4) containing 2 mg of lysozyme per ml (Worthington Biochemical Corp.). Samples were frozen and thawed four times in an alcohol-dry ice mixture, incubated for 2 min at 37 C, frozen and thawed once more, incubated at 37 C for 1.5 min, and then brought rapidly to 0 C. DNA was digested in the crude extract by addition of 1 drop of 1 M $MgSO_4$ and 100 μg of electrophoretically purified deoxyribonuclease for 5 min at 0 C. At that time, 0.5 ml of 0.5 M ethylene-diaminetetraacetic acid (EDTA) and 0.3 ml of 20% sodium dodecyl sulfate (SDS) were added. The extracts were allowed to remain at room temperature for 30 min, and were then centrifuged at 10,000 $\times$ *g* for 5 min. The supernatant solution was layered on 15 to 30% sucrose gradients containing 0.1 M NaCl-0.01 M Tris-0.01 M EDTA-0.2% SDS (*p*H 7.4). Gradients (36 ml) were centrifuged in a Spinco SW27 rotor in an L2-65B ultracentrifuge at 26,000 rev/min for 17 hr at 25 C. The gradients were fractionated by inserting a stainless-steel needle at the bottom of the tube and were analyzed with a Gilford model 240 spectrophotometer. Radioactivity in the samples was measured by filtering the fractions through type HA filters (Millipore Corp.) after precipitation with 5% trichloroacetic acid in the presence of 1 drop of 0.2% bovine serum albumin.

Isolation of Messenger RNA

Messenger RNA was labeled by the addition of 20 μc of tritiated uridine per ml (29 c/mmole; Nuclear-Chicago Corp., Des Plaines, Ill.) to 10 ml of bacteria growing in minimal medium. Uptake was limited to 1 min. The bacteria were poured on saline-azide ice and centrifuged at 12,000 $\times$ *g* for 10 min at 0 C. The pellets were suspended in 5 ml of 0.15 M sodium chloride-0.01 M Tris-0.005 M $MgSO_4$ (*p*H 7.0). The cells were ruptured by passing through a French press at 2 C (12,000 to 16,000 lb total load). Digestion with deoxyribonuclease was carried out as described above. After addition of 0.5 ml of 0.5 M EDTA and 0.3 ml of 20% SDS, the samples were extracted with an equal volume of water-saturated redistilled phenol at 60 C for 5 min. After low-speed centrifugation (4,000 $\times$ *g*; 5 min), the supernatant solution was made 0.25 M with respect to sodium chloride, and the RNA was precipitated with 3 volumes of 95% ethyl alcohol at -20 C. Samples were stored for at least 1 hr at -20 C and were centrifuged at 12,000 $\times$ *g* for 30 min. The RNA pellets were resuspended in 2$\times$ SSC (SSC is 0.15 M NaCl-0.015 M sodium citrate). RNA samples were dialyzed twice overnight against 100 volumes of 2$\times$ SSC and stored at -20 C.

DNA-RNA Hybridization

DNA-RNA hybrids were formed by annealing heat-denatured DNA (20 μg) with RNA in 2$\times$ SSC for 12 hr at 60 C, and were detected by the method of Nygaard and Hall (14). The annealed material, 0.5 ml, was treated with 0.5 ml of a 0.01 M NaCl solution containing 10 μg of pancreatic ribonuclease per ml and 10 units of T1 ribonuclease per ml (Worthington Biochemical Corp.; both enzymes were heated at 80 C for 10 min to eliminate deoxyribonuclease activity) for 30 min at 37 C. The material was then diluted fivefold with 4$\times$ SSC and filtered on membrane filters, type HA (Millipore Corp.). The filters were soaked in 4$\times$ SSC for at least 10 min prior to use. The material retained on membrane filters was washed with 50 ml of 4$\times$ SSC, and the filters were dried and counted as described above.

RESULTS

Figure 1 shows the results of an experiment designed to determine the amount of ^{3}H-thymidine incorporated into phage and host DNA. *B. subtilis* 168-2 was infected with a multiplicity of five phages per bacterium, and 2.5 μc of ^{3}H-thymidine/ml (8 c/mmole) was added. At various time intervals, samples of infected cells were

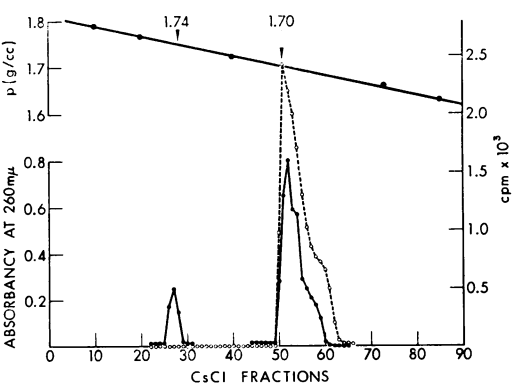

FIG. 1. *Distribution of ^{3}H-thymidine in host and viral DNA. B. subtilis 168-2 was infected (multiplicity of infection = 5) with phage 2C in synthetic medium. ^{3}H-thymidine (2.5 μc/ml) was added along with the phage. The total DNA content of the bacteria was extracted 30 min after infection (see Materials and Methods). The DNA was sedimented in a CsCl gradient in an SW39 rotor (33,000 rev/min; 60 hr; 25 C). The density of viral DNA in CsCl is 1.742 g/cc, whereas B. subtilis DNA forms a band at 1.703 g/cc. Symbols: ●, optical density at 260 mm; ○, radioactivity.*

chilled rapidly, and the total DNA content was extracted by the method of Marmur (12). Phage and bacterial DNA were separated by preparative CsCl density gradient centrifugation (13), and the distribution of radioactivity and ultraviolet (UV)-absorbing material was determined. The data presented in Fig. 1 show that 30 min after phage addition ^{3}H-thymidine was incorporated exclusively into host DNA. Similar results were obtained with DNA samples isolated throughout the latent period. The specific activity of the host DNA did not, however, increase late in infection. Incorporation of ^{3}H-thymidine measured, therefore, host DNA synthesis. The entrance of label into host DNA without an increase in specific activity suggested that host DNA synthesis did not stop immediately after phage infection. To test this possibility, experiments were performed to measure the rate of host DNA synthesis in phage-infected cells at various times after infection. *B. subtilis* 168-2 cells infected in minimal medium with phage 2C (multiplicity of infection = 5) were pulsed with ^{3}H-thymidine (2.5 μc/ml) for 3 min at 3-min intervals. Uptake of label was terminated by addition of cold 5% trichloroacetic acid containing 2.5 mg of nonradioactive thymine per ml. The samples were allowed to stand at 0 C for 2 hr, filtered, and washed with 5% trichloroacetic acid on membrane filters. Filters were dried and counted. As shown in Fig. 2, the rate of host DNA synthesis increased initially and then rapidly decreased 6 min after phage infection. Addition of chloramphenicol (100 μg/ml) along with virus did not affect the rate of uptake of radioactive thymidine, indicating that protein synthesis is necessary to arrest host DNA synthesis.

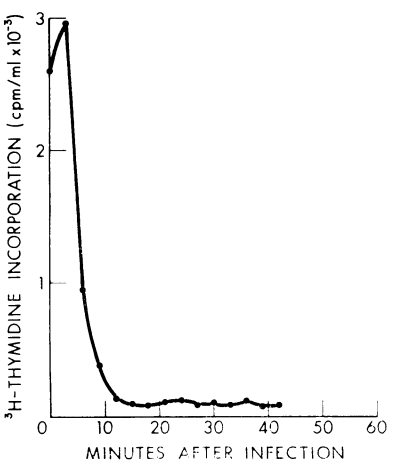

FIG. 2. *Rate of host DNA synthesis in 2C-infected B. subtilis 168-2. Samples of infected bacteria (multiplicity of infection = 5) were pulsed with ^{3}H-thymidine (2.5 μc/ml) for 3 min at 3-min intervals. The samples were processed as described in Materials and Methods.*

TABLE 1. *Biological activity of host DNA in 2C-infected B. subtilis*[a]

Time after phage addition (min)	Proline-independent transformants per μg of DNA/per ml
Uninfected	8.4×10^4
10	7.9×10^4
20	8.7×10^4
35	8.0×10^4
50	7.3×10^4

[a] The biological activity of host DNA was assayed after infection with phage 2C at a multiplicity of infection of 5.

To determine whether the arrest of host DNA synthesis is also followed by a degradation of the bacterial DNA, the biological activity of host DNA extracted at various times during the latent period was measured. *B. subtilis* 168-2 (ind$^-$ leu$^-$) DNA from phage-infected cells was added to competent cells of *B. subtilis* 168-3 (ind$^-$ pro$^-$) at a final concentration of 1 μg/ml. The number of proline-independent transformants was determined after 30 min of contact with DNA. Controls were performed by using *B. subtilis* 168-2 DNA from uninfected cells. These experiments showed that the biological activity of host DNA does not change appreciably during the latent period of the bacteriophage (Table 1). Similar results have been obtained with other HMU-containing bacteriophages of *B. subtilis* (17).

The rate of ribosomal RNA synthesis in normal and phage-infected *B. subtilis* 168-2 was measured at various multiplicities as outlined in MATERIALS AND METHODS. The data given in Table 2 indicate that in phage 2C-infected *B.*

TABLE 2. *Rate of synthesis of ribosomal RNA in 2C-infected B. subtilis*[a]

Sample	Mutiplicity of infection	Specific activity (counts per min per optical density unit)	
		23*S*	16*S*
Uninfected	—	1,540	1,850
Infected	2.5	1,710	1,920
Uninfected	—	2,460	2,320
Infected	10.7	2,520	3,160

[a] At 30 min after infection, cultures of *B. subtilis* 168-2 were pulsed with ^{14}C-uridine (0.1 μc/ml) for 2 min, and chased with actinomycin (5 μg/ml) and cold uridine for 6 min. Ribosomal RNA was isolated and fractionated on sucrose gradients.

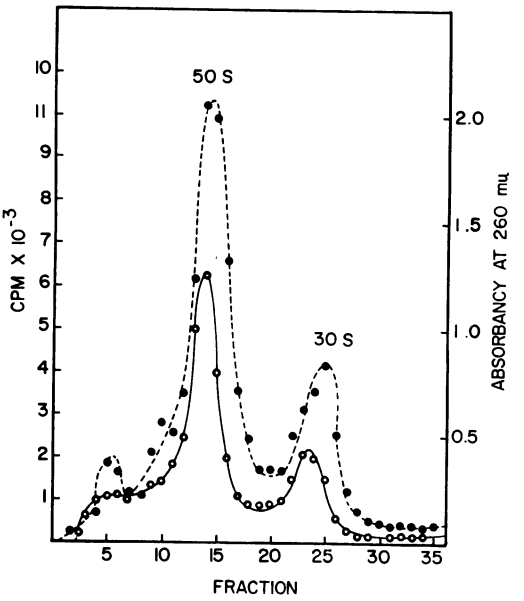

FIG. 3. *Distribution of ribosomal RNA from infected B. subtilis in ribosomal subunits. B. subtilis A26U⁻ (100 ml; 7 × 10⁷/ml) was infected in minimal medium with phage 2C (multiplicity of infection = 5) and incubated for 20 min at 37 C. The culture was pulsed with 1 μc of ³H-uridine per ml for 2 min in the presence of 20 μg/ml of nonradioactive uridine. Actinomycin D (5 μg/ml) and uridine (0.5 mg/ml) were added, and incubation was continued for 10 min. The culture was poured on azide-ice, centrifuged, washed in 0.01 M Tris-0.06 M KCl-0.01 M $MgCl_2$ (pH 7.4) and resuspended in 5 ml of that buffer. Cells were disrupted in a French press, and the extract was digested with 100 μg of electrophoretically purified deoxyribonuclease for 5 min at 0 C. Brij 58 was added at a final concentration of 0.06%. After low-speed centrifugation to remove debris, the extract was centrifuged at 50,000 rev/min for 2 hr at 2 C in a Spinco type 65 rotor. The pellet was suspended in 2 ml of 0.01 M Tris-0.06 M KCl-0.005 M $MgCl_2$ (pH 7.4). A 1-ml amount was layered on a 15 to 30% sucrose gradient (36 ml) buffered with 0.01 M Tris-0.06 M KCl-0.005 M $MgCl_2$ (pH 7.4). The ribosomal subunits were separated by centrifugation in a Spinco SW27 rotor at 24,000 rev/min for 16 hr at 2 C. Symbols; ○, optical density at 260 nm; ●, radioactivity.*

subtilis ribosomal RNA continues to be synthesized late in infection. Similar results were obtained at early times during the latent period.

Ribsomal RNA synthesized by infected bacteria enters ribosomal 30*S* and 50*S* subunits, as can be seen from the data presented in Fig. 3. Similar results were obtained throughout the viral latent period.

To determine whether viral infection could affect the synthesis of host-specific proteins, the inducibility of sorbitol dehydrogenase was meas-

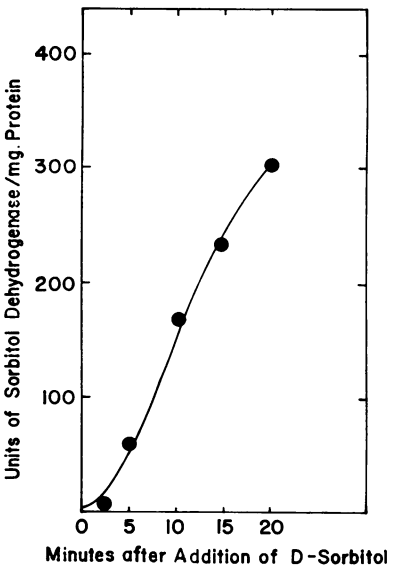

FIG. 4. *Induction of D-sorbitol dehydrogenase in B. subtilis. D-Sorbitol dehydrogenase was induced and detected as described in Materials and Methods.*

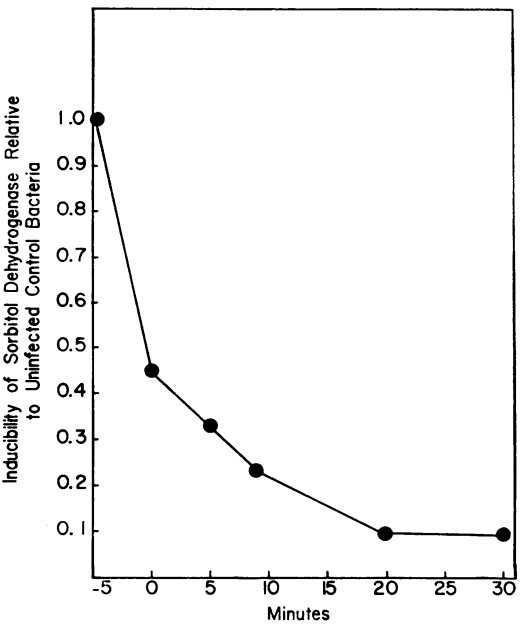

FIG. 5. *Inducibility of D-sorbitol dehydrogenase in 2C-infected B. subtilis. The inducibility of D-sorbitol dehydrogenase was measured in infected bacteria (multiplicity of infection = 5) at various times after infection (see Materials and Methods). The results are plotted as amount of enzyme detected relative to uninfected control cultures.*

TABLE 3. *Hybridization of host and virus-specific pulse-labeled RNA in phage-infected B. subtilis in the presence and absence of chloramphenicol*[a]

Multiplicity of infection	Chloramphenicol added	Pulse-labeled RNA forming hybrid with		Fraction of pulse-labeled RNA hybridizing with host DNA relative to uninfected controls
		Marburg DNA	2C DNA	
	μg	%	%	
2.3	0	4.7	35.0	0.45
10.7	0	2.6	31.7	0.23
Uninfected	0	10.4	0.01	1.0
2.3	100	6.4	21.5	0.62
10.7	100	3.2	32.4	0.31
Uninfected	100	10.3	0.005	1.0

[a] Messenger RNA was isolated and hybridized with *B. subtilis* Marburg and phage 2C DNA as described in Materials and Methods. The ratio of DNA to RNA was greater than 50 in all cases to ensure an excess of binding sites and prevent saturation of the DNA.

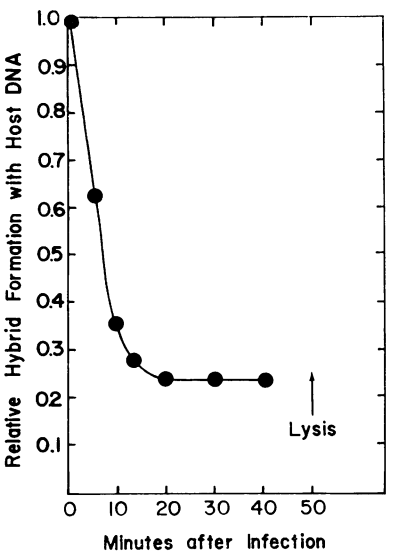

FIG. 6. *Synthesis of host messenger RNA in 2C-infected B. subtilis. Messenger RNA was isolated from infected B. subtilis (multiplicity of infection = 5) at various times during the latent period and hybridized with host DNA (see Materials and Methods). The results are presented as the fraction of pulse-labeled RNA hybridizing with host DNA relative to uninfected control bacteria.*

ured at various times after infection. Sorbitol dehydrogenase can be induced in *B. subtilis* as described in MATERIALS AND METHODS. Figure 4 shows the rate and time of appearance of the enzyme in uninfected bacteria after addition of 1% sorbitol. The data in Fig. 5 show that the induction of sorbitol dehydrogenase in phage-infected *B. subtilis* 168-2 is considerably impaired. These data suggested that viral infection could affect the synthesis of at least some classes of messenger RNA in its host.

Correlated with the inability of bacteria to synthesize sorbitol dehydrogenase is a depression of host messenger RNA synthesis. The fraction of pulsed labeled radioactivity hybridizing with host and viral DNA was measured at various multiplicities, in the presence or absence of chloramphenicol, in normal and phage-infected bacteria. The results presented in Table 3 show that phage 2C depresses the synthesis of host messenger RNA to the extent of 75 to 80%. The depression of host messenger synthesis is dependent on the multiplicity of infection and occurs in the presence of chloramphenicol, which presumably prevents the synthesis of phage-specific proteins.

Figure 6 shows the time course of the arrest of host messenger RNA synthesis in bacteria infected with phage 2C at a multiplicity of five phages per bacterium.

DISCUSSION

In *B. subtilis* infected with bacteriophage 2C host DNA synthesis stops shortly after infection. This process apparently requires protein synthesis and the appearance of phage-specific products. A similar situation has been found in *B. subtilis* SP8 (17) and φe (7). In the latter case, host DNA synthesis is arrested in part by the action of a phage-specific protein which combines with and inactivates thymidylate synthase in an irreversible fashion. After the arrest of host DNA synthesis in phage 2C-infected *B. subtilis*, host DNA is not degraded to fragments smaller than transforming DNA. Similar conclusions have been reached in experiments with *B. subtilis* infected with SP8 (17). This process is different than the extensive degradation of host DNA which occurs after infec-

tion with T-even phages infecting *Escherichia coli* (4).

Infection of *B. subtilis* with phage 2C allows the continuation of ribosomal RNA synthesis. It is difficult to recover ribosomal RNA in cells infected at high multiplicities late in infection since bacteria begin to lyse and release some of their RNA content into the medium. For this reason, optical density patterns of RNA isolated from bacteria late in infection do not show a typical 2:1 ratio of 23*S* to 16*S* RNA. At high multiplicities, late in infection, calculation of the specific activity of 23*S* and 16*S* RNA (especially 16*S* RNA) has given values which have always been higher than those for 23*S* and 16*S* RNA from control, uninfected bacteria. Preliminary experiments have indicated that over 55% of phage messenger RNA sediments in the 12*S* to 18*S* region of sucrose gradients. It is possible that the higher specific activity found for bacterial 16*S* RNA in cells infected at high multiplicities may represent messenger RNA which is not completely degraded during the actinomycin chase.

Ribosomal RNA continues to be synthesized and enters ribosomal subunits in infected bacteria, but host protein synthesis is drastically affected. By 10 min after infection the bacteria's capacity to synthesize sorbitol dehydrogenase is reduced by 80%. Cessation of host protein synthesis is correlated with a depression of host messenger RNA synthesis. Messenger RNA synthesis in infected bacteria is reduced by 75 to 80%. This effect is dependent on multiplicity and occurs in the presence of chloramphenicol, which, at the concentration used, prevents over 98% of protein synthesis.

Levinthal et al. (9) reported a reduction of host protein synthesis and normal ribosomal RNA synthesis in SPO1-infected *B. subtilis*. These observations undoubtedly represent an effect similar to the one reported here. In λ- and T4-infected *E. coli*, all RNA and protein synthesis is depressed in a multiplicity-dependent manner; this effect also results in the presence of chloramphenicol (26, 27). In these cases, depression of all RNA species occurs equally.

Infection of *B. subtilis* with a virulent phage such as 2C results, therefore, in a modification of the transcription of the host chromosome. The synthesis of the products of ribosomal RNA cistrons is apparently normal early and late in the virus cycle, whereas messenger RNA synthesis is severely depressed. Phage C2 is unable to stop host messenger RNA synthesis completely since it appears that approximately 20% of *B. subtilis* messenger RNA molecules are still synthesized in infected bacteria. Some of the host genes such as ribosomal RNA cistrons, therefore, escape the control imposed on messenger RNA by the virus. Virus-induced modification of the transcription of certain host genes in the presence of chloramphenicol could involve restriction by phage messenger RNA of the action of RNA polymerase to certain regions of the bacterial chromosome (27). Modification of RNA polymerase specificity could occur either by a direct action of phage messenger RNA on the chromosome or through its association with the polymerase. In infected bacteria residual host messenger RNA synthesis may represent the normal transcription of genes unaffected by this control mechanism. The clustering of these genes on the bacterial chromosome could make it possible for a single target to be affected by a virus-specific messenger RNA, or, through its association with the polymerase, the messenger RNA might modify the transcription of a large region distal to the segment which is transcribed normally.

Ribosomal RNA cistrons in *B. subtilis* are clustered near the origin of the chromosomes (15). It is possible that this particular arrangement might play a role in the mechanism described here. The genes specifying messenger RNA molecules which are still made by infected bacteria might be clustered in this region, and transcription distal to the segment of the chromosome might be impaired through the action of either a phage messenger RNA or a viral internal protein associating with the host chromosome at a specific region.

Work in progress is designed to discover whether genes specifying residual host messenger RNA species in infected bacteria are clustered near the origin of the bacterial chromosome, or to determine whether all host messenger RNA species are synthesized at reduced rates in infected bacteria.

ACKNOWLEDGMENTS

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