JOURNAL OF VIROLOGY, Dec. 1974, p. 1482-1493
Vol. 14, No. 6

Printed in U.S.A.

Origin and Metabolic Properties of the RNA Species Formed During the Replication Cycle of Virus 2C

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Received for publication 29 July 1974

When short pulses of [^{3}H]uracil were administered to *Bacillus subtilis* infected with phage 2C, the main species of labeled RNA was a 10*S* component that hybridized chiefly, but not exclusively, with the heavy strand of 2C DNA. After long pulses, most of the radioactivity was found in the 23*S*, 16*S*, and 5*S* rRNA's, which are coded for by the cell genome. Formation of such RNA species was reduced but not suppressed upon infection, the extent of inhibition being proportional to the virus-to-cell ratio. When bacteria were incubated with virginiamycin, an inhibitor of protein synthesis, and then infected with phage 2C, formation of virus-specific RNA decreased. This antibiotic also reduced the preferential transcription of the heavy strand of 2C DNA. The methylation pattern of rRNA remained unchanged upon infection with phage 2C. Virginiamycin reduced both the methylation and stability of rRNA in uninfected cells; this effect, however, was clearly reduced during the viral cycle. It can be concluded that in 2C-infected *B. subtilis*, cellular and viral RNA species are simultaneously synthesized and a preferential transcription of viral message depends not only on the number of available copies of viral template, but also on their translation. Moreover, virus-dictated proteins are responsible for the inhibition of cellular RNA formation as well as for the asymmetrical transcription of phage genome. Finally, virginiamycin and phage 2C have antagonistic, nonoverlapping effects on the metabolism and function of the RNA of the host cell.

Antibiotics have been extensively used to dissect the metabolic pathways of virus-infected cells and to clarify the mechanism of viral replication. Thus, addition of inhibitors of protein synthesis at different moments of the viral cycle has allowed the characterization of various classes of virus-specific proteins (11) and has helped the fractionation of mRNA molecules that are transcribed from the early and late cistrons of the viral genome (29).

Among the antibiotics, virginiamycin has already proved an exceptional tool in that it contains two components (M and S) displaying a synergistic inhibitory action on sensitive microorganisms (4, 36). The activity of a mixture of M and S is not equivalent to those of single components. In bacteria, each virginiamycin factor increases by 100-fold the growth-inhibiting power of its partner (6), whereas in algae it is the inhibitory activity of M that is rendered permanent by S (8, 37, 38; C. Cocito and M. Shilo, submitted for publication). Previous publications have shown that virginiamycin M acts on the 50*S* ribosomal subunits and blocks the elongation of polypeptide chains in vitro and in vivo (7, 9).

In the present work, virginiamycin was used to study the mechanism of phage 2C replication in *Bacillus subtilis*. This virus bears some morphological and biochemical resemblances to the well-known T-even phages that multiply in *Escherichia coli* (21, 35). However, unlike T4, virus 2C does not completely block the formation of cellular macromolecules (5).

Experiments described here mainly concern the properties and metabolism of different RNA species that are labeled in 2C-infected *B. subtilis*. Formation of polyribosomes and ribosomes during the viral cycle will be analyzed in another paper (C. Cocito, submitted for publication).

MATERIALS AND METHODS

Bacterial and viral strains. The wild-type strain 168/6 of *B. subtilis* and two of its mutants, 168/2 (*leu*$^-$ *tryp*$^-$) and A26 (*uracil*$^-$ *tryp*$^-$), were used in different experiments. Composition of the growth media has been previously reported (4). Techniques for produc-

tion, purification, and titration of phage 2C in *B. subtilis* 168 were described in an earlier publication (5). Buoyant density of this virus in CsCl was ρ_{23} = 1.52 g/cm³. Biophysical properties of 2C can be found elsewhere (21, 35). The strain used was isolated by J. Pène and obtained through the courtesy of J. Marmur (Albert Einstein College, Yeshiva University, Bronx, N.Y.).

Isolation of viral DNA and strand separation. Suspensions of purified viral particles in SSC (0.15 M NaCl + 0.015 M trisodium citrate, pH 7.2) were mixed with freshly redistilled phenol previously saturated with the same buffer. The aqueous layers were extracted with ether, and DNA was precipitated with 3 volumes of ethanol and stored under ethanol at −18 C. Denaturation of native DNA (2 units of absorbancy at 260 nm [A_{260}] per ml in 10-fold-diluted SSC) was achieved by heating nucleic acid solutions for 5 min in a boiling water bath and then quickly transferring them to melting ice. The two strands of viral DNA were separated either by equilibrium centrifugation CsCl (ρ_{23} = 1.75 g/cm³, 72 h, 23 C, 33,000 rpm) or by fractionation of methylated albumin-kieselguhr columns (19). Apparent buoyant densities in CsCl were 1.744 g/cm³ for native DNA, 1.762 g/cm³ for the heavy strand, and 1.752 g/cm³ for the light strand. Native viral DNA had an A_{260} = 0.02 cm²/μg, a T_m = 78 C, and a guanine-plus-cytosine value of about 39%.

Fractionation and analysis of RNA. Host cells labeled with long and short pulses of radioactive precursors were suspended in SAAS (0.1 M NaCl, 0.01 M sodium dodecyl sulfate, 0.05 M sodium acetate-acetic acid buffer, pH 5.2) and disrupted by the compression-decompression with a French press. The homogenates were extracted with equal volumes of freshly redistilled water-satrurated phenol, and RNA was precipitated with 3 volumes of ethanol at −18 C. For ultracentrifugal fractionation, solutions of RNA in SAAS were layered over 15 to 30% sucrose gradients in SAAS and centrifuged for 1 day at 23,000 rpm in a Spinco 25.1 swingout rotor at 23 C. In some instances, sodium dodecyl sulfate homogenates were fractionated without previous phenol extraction. Scanning of gradients at 260 nm was carried out in a Gilford spectrophotometer, and collected fractions were treated with 0.5 M trichloracetic acid at 4 C for 1 h before filtration through 0.4-μm membrane filters, which were counted in a scintillation counter (4). Chromatographic fractionation of RNA was carried out on methylated albumin-kieselguhr columns (19) with sodium phosphate buffers, pH 5.2, of different molarities: 0.1 M for loading, 0.3 M for washing, and a 0.3 to 1.0 M gradient for elution (2, 3). Eluates were scanned with a Gilson monocromator at A_{260}, and trichloroacetic acid-precipitated nucleic acids were collected on membrane filters and counted.

Hybridization techniques. Hybridization on solid supports was carried out as follows. Solutions of either denatured DNA or of single strands containing 0.2 A_{260} units per sample, in fourfold-concentrated SSC, were filtered through 0.4-μm filters (Millipore Corp., Bedford, Mass.), which were washed and dried for 4 h at 20 C and then for 2 h at 80 C in vacuo. The hybridization mixture contained: one DNA filter; different portions of labeled RNA; 0.50 M NaCl, 0.25 M sodium citrate-citric acid buffer, pH 7.8; and 0.05% phenol. Annealing was carried out in sealed vials, which were kept for 4 to 14 h at 56 C in a shallow water bath. Filters were then incubated with 5 μg of RNase in 0.05 M Tris-hydrochloride buffer, pH 7.6, for 5 min at 37 C, washed, dried, and counted. For each sample, a blank containing the same amount of labeled RNA but no DNA was made, and the corresponding counts were subtracted. This technique is close to the procedure originally described by Nygaard and Hall (26) and by Gillespie and Spiegelman (13).

Hybridization and competition experiments were also carried out with DNA solutions replacing DNA filters. In this case, at the end of the incubation period, reaction mixtures were filtered on presoaked filters, which were subsequently washed, incubated with RNase, dried, and counted.

RESULTS

Formation of RNA in virginiamycin-treated, virus-infected cells. Infection of *E. coli* with phages T2 and T4 produces a sudden halt of RNA formation (10); this inhibition, however, does not take place when the cells are incubated with chloramphenicol (24). To find out whether a similar situation occurred under our experimental conditions, *B. subtilis* was infected with virus 2C in the presence of virginiamycin. Different samples were labeled with [³H]uracil for 15 min during either the eclipse phase or the early and late maturation phases. Cells harvested by centrifugation were disrupted, and the extracted RNA was fractionated by ultracentrifugation in density gradients.

23*S*, 16*S*, and 5*S* plus 4*S* RNAs were the main species labeled in uninfected cells after 15-min pulses (Fig. 1). A similar pattern was found at early times after infection with 2C (Fig. 1B). Formation of rRNA, however, progressively decreased as the multiplication cycle progressed and, in addition, a large portion of the RNA labeled after infection sedimented in the 10*S* region. Ultracentrifugal patterns from cells that were infected in the presence of virginiamycin showed little or no 23*S* rRNA and a high 10*S* to 4*S* radioactivity peak (Fig. 1C); such a pattern is similar to that previously observed in uninfected cells that were incubated with the antibiotic (4).

Quantitative data concerning experiments of this type (fractions from density gradients were pooled according to the schema of Fig. 1A) indicated that the formation of rRNA as well as the ratio of high- to low-molecular-weight RNA progressively decreased during the viral cycle as compared with the controls (Table 1). These

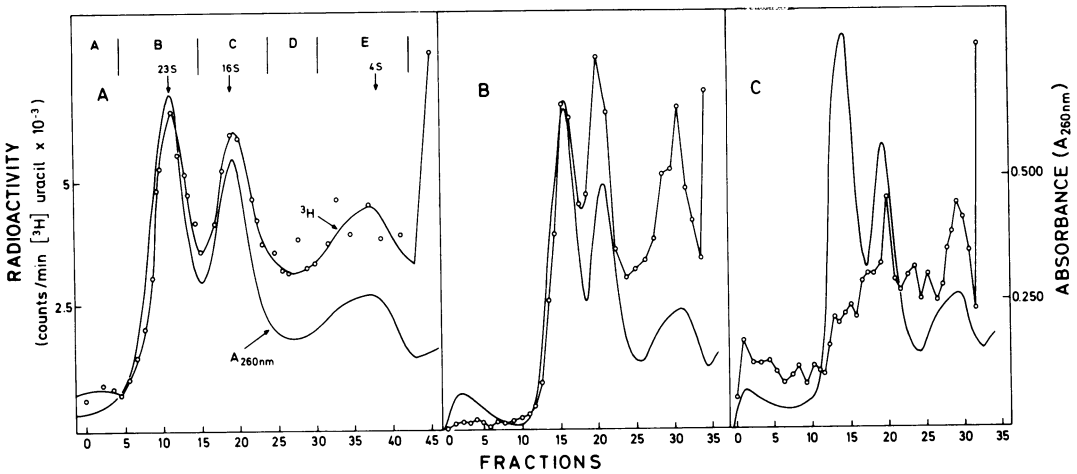

FIG. 1. *RNA species labeled after infection with virus 2C. B. subtilis 168/6 was infected with phage 2C (multiplicity of infection = 30) in the presence and in the absence of virginiamycin (2.5 μg of M and S per ml) and labeled for 15 min with [6-³H]uracil (12 Ci/mmol, 2 μCi/ml) 2, 15, and 30 min after infection. Cell homogenates were incubated with DNase (1 μg/ml, 23 C, 5 min) and extracted with sodium dodecyl sulfate and phenol. RNA was fractionated by density gradient centrifugation as described in Materials and Methods. (A) Control, uninfected cells, no virginiamycin; (B and C) phage-infected cells, labeled from 2 to 17 min either in the absence (B) or presence (C) of virginiamycin.*

TABLE 1. *RNA biosynthesis during the viral cycle*

Virginiamycin		Virus[a]	Labeling (min)	Radioactivity incorporated into RNA[b]						Ratio HMW RNA/LMW RNA[c]
Factor	Amt (μg/ml)			>23S	23S	16S	10S	4S	<4S	
		−	2-17	2.92	25.17	19.82	13.05	25.41	10.30	1.77
		+		2.91	57.17	40.84	28.78	55.24	7.63	1.77
M	50	+		13.77	54.31	29.77	27.73	66.51	9.17	1.26
S	50	+		10.70	20.19	22.52	23.36	47.42	8.39	0.90
MS	2.5	+		5.92	71.80	31.05	36.06	86.03	8.03	1.19
		−	14-29	5.64	47.81	30.80	27.42	39.17	9.83	2.01
		+		3.90	28.35	29.43	21.85	74.31	6.22	0.78
M	50	+		19.67	27.29	23.37	19.20	21.87	3.97	2.32
S	50	+		20.25	8.62	24.61	35.49	23.15	5.93	1.44
MS	2.5	+		7.82	12.34	18.37	8.73	17.98	3.23	1.71
		−	22-37	3.15	71.32	48.08	24.28	46.30	15.58	2.58
		+		1.59	20.32	29.02	10.61	133.69	11.98	0.37
M	50	+		13.04	29.22	46.61	16.15	44.52	11.11	1.70
S	50	+		11.78	23.80	33.31	30.74	32.18	3.34	1.77
MS	2.5	+		12.92	21.97	47.04	34.16	35.87	7.43	1.92

[a] Infection at time zero with 10 PFU of virus 2C per cell.
[b] Acid-insoluble counts per minute × 10^{-3} of [³H]uracil/ml.
[c] HMW, High molecular weight; LMW, low molecular weight.

inhibitory effects produced by the virus were reduced in the presence of virginiamycin (with the exception of 23S rRNA).

Thus, unlike the T2 *E. coli* system, formation of rRNA and tRNA continued during the entire replication cycle of 2C in *B. subtilis*. Moreover, in virginiamycin-treated, virus-infected cells, the two metabolic patterns characteristic of the virus and virginiamycin, respectively, were both evident but less pronounced.

Action of virginiamycin on the methylation of RNA in the host cells. It was previously

reported (4) that methylation of rRNA was strongly inhibited by virginiamycin; the undermethylated RNA thus formed was metabolically unstable and underwent turnover. Does such inhibition still occur after viral infection?

Exponential cultures of *B. subtilis* were incubated with virginiamycin, infected with phage 2C, and labeled for 8 min with [^{3}H]uracil as a specific RNA marker and with [*methyl*-^{14}C]methionine as methyl group donor. Labeled cells were chased for 10 min with a mixture of unlabeled uracil and methionine. Portions of infected cells were labeled either during the eclipse phase or during the early and late maturation phases, and samples were taken at the end of both labeling and chasing periods. After cell disruption, RNA was extracted and fractionated in density gradients, and the ^{14}C/^{3}H ratio of the fractions was determined to assess the degree of methylation of RNA during the viral cycle.

Patterns of RNA methylation shown in Fig. 2A and 2B are from uninfected cells that were labeled and chased in the absence of virginiamycin. From these pictures, those of uninfected virginiamycin-treated cells differed in three respects: (i) the peak of 23*S* rRNA was virtually absent; (ii) the level of methylation was low, as indicated by the ^{14}C/^{3}H ratio; and (iii) there was a turnover of rRNA as shown by a reduction of this ratio upon chasing (4).

In virus-infected cells, 23*S* and 16*S* rRNA formation was reduced, but the degree of methylation was unaffected during the eclipse phase (Fig. 3A and B) as well as during the maturation phase (Fig. 3C and D). After incubation of virus-infected host cells with virginiamycin (Fig. 2C and D), the peak of 23*S* rRNA was reduced but still visible and the level of methylation was low, yet its value was higher than that of uninfected virginiamycin-treated cells (4).

The rate of uracil and methyl group incorporation into 23*S* and 16*S* rRNA progressively decreased as the replication cycle progressed, but the ratio of ^{14}C to ^{3}H in infected bacteria was similar to or even higher than that of the controls (Table 2). On the other hand, in virginiamycin-treated host cells, very low ^{14}C/^{3}H values were found, irrespective of the time of virginiamycin addition (either the beginning or the end of the eclipse phase); however, such undermethylated RNA was apparently stable.

It was evident, therefore, that infection with virus 2C decreased the formation of rRNA without affecting its methylation and stability, whereas virginiamycin specifically reduced the methylation and stability of RNA. In addition, viral infection reduced the effect of virginiamycin on RNA metabolism.

Influence of the multiplicity of infection on the metabolism and methylation of RNA. Since the replication cycles of many viruses show different metabolic patterns at increasing multiplicities of infection, synthesis and methylation of RNA were followed at different virus-to-cell ratios.

Indeed, the inhibition of rRNA formation during the viral cycle was directly proportional to the multiplicity of infection, and this effect was not abolished by virginiamycin (Table 3). Moreover, the inhibition of rRNA methylation due to the antibiotic was reduced after infection with 2C. In fact, as the multiplicity of infection increased, the ratio of ^{14}C to ^{3}H in RNA showed a parallel enhancement.

It can be concluded that inhibition of RNA formation is a basic expression of virus development; the degree of such inhibition is in fact proportional to the number of copies of viral template. An additional consequence of the infection with 2C is the reduction of virginiamycin-dependent inhibition of RNA methylation. Consequently, the phage and the antibiotic exert antagonistic, nonoverlapping effects on cell metabolism.

Cell- and virus-specific RNA formation during the multiplication of phage 2C. Experiments described in the previous sections showed the high heterogeneity of RNA made during the replication cycle. They did not indicate, however, the origin of the different RNA species. To solve this problem, phage-infected cells were labeled with short and long pulses of [^{3}H]uridine. The extracted RNA was hybridized with *B. subtilis* and 2C DNA, and the percentage of hybridization was determined (Table 4). Clearly, a large portion of the RNA that was labeled by short pulses during the entire replication cycle was coded for by the viral genome. Moreover, the percentage of hybridization of labeled RNA with 2C DNA was lower after long pulses than after short pulses. A treatment with viriginiamycin reduced the formation of early viral messenger and occasionaly increased the amount of RNA hybridizing with host DNA.

This indicates that the viral template is preferentially transcribed with respect to the cell genome. This priority effect depends on some virginiamycin-sensitive translational events.

Characterization of RNA synthesized on the viral template. Hybridization with cellular and viral DNA of RNA fractions obtained by

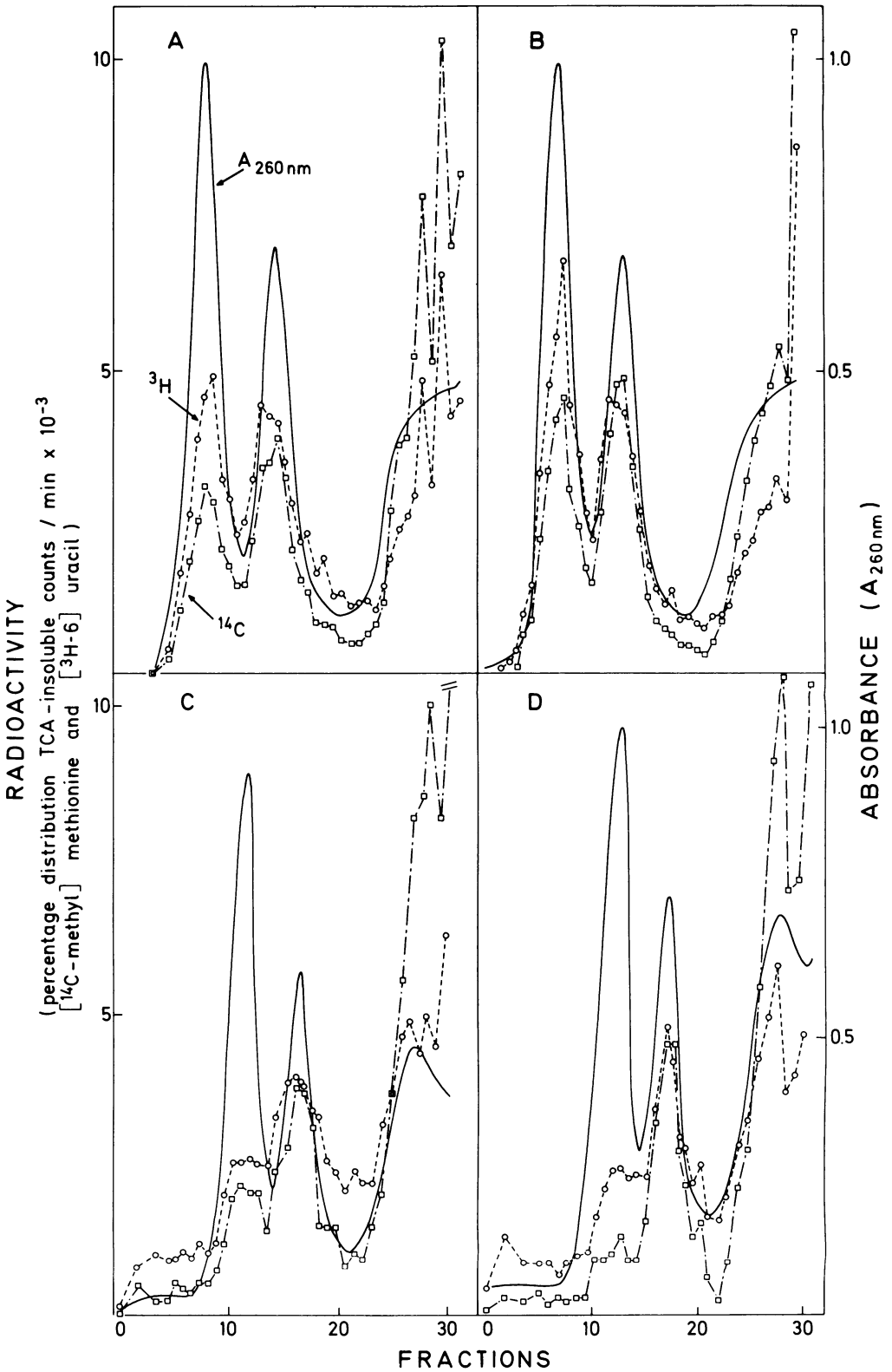
A
B
C
D
A 260 nm
^{3}H
^{14}C
RADIOACTIVITY
(percentage distribution TCA-insoluble counts / min x 10^{-3}
[^{14}C-methyl] methionine and [^{3}H-6] uracil)
ABSORBANCE ($A_{260\,nm}$)
FRACTIONS
0
10
20
30
5
10
0.5
1.0

FIG. 2.

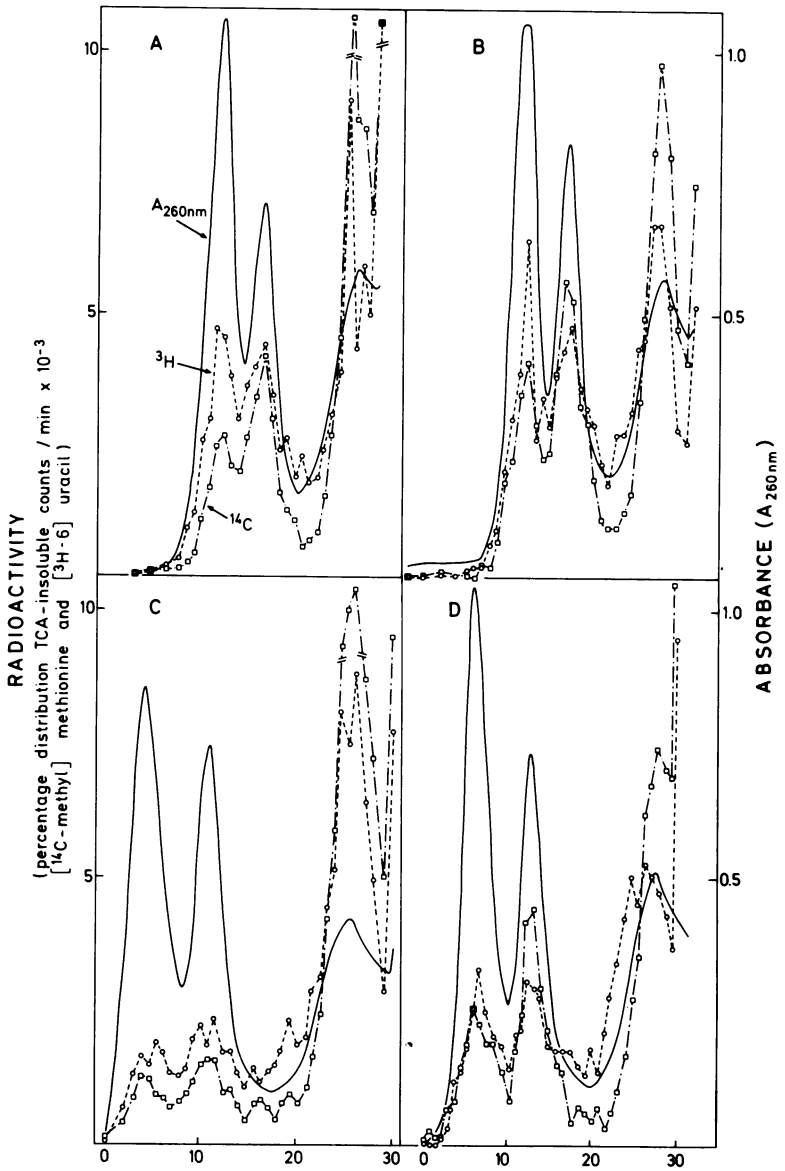

FIG. 3.

FIG. 2 AND 3. *Methylation and stability of RNA in virus-infected, virginiamycin-treated cells. B. subtilis A26, infected with phage 2C (multiplicity of infection = 50) either in the presence or absence of virginiamycin (5 μg of M and S per ml), was labeled for 8 min with a mixture of [6-^3H]uracil (10 Ci/mmol, 5 μCi of ^3H per 40 μg of ^1H per ml) and [methyl-^{14}C]L-methionine (24.6 mCi/mmol, 0.5 μCi/ml) and chased for 10 min with [^1H]uracil (1.7 mM) and [^{12}C]methionine (0.33 mM). RNA extracted from the homogenates of labeled and chased cells was fractionated by centrifugation in density gradients. (2A and B) Control (no virus, no antibiotic), labeled (A) and chased (B) samples. (2C and D) Virginiamycin-treated virus-infected cells, labeled ([C] 0 to 14 min) and chased ([D] 14 to 24 min) samples. (3 A–D) Virus-infected cells (no antibiotic). (A) Labeled during the eclipse phase (5 to 13 min); (C) Labeled during the maturation phase (17 to 25 min); (B and D) chased samples.*

centrifugation in density gradients may furnish additional information about the RNA produced at different times after infection. Moreover, the origin of the genetic information for viral messenger could be identified with more precision by annealing the RNA isolated from virus-infected cells with single strands of 2C DNA.

TABLE 2. *Methylation of RNA in virginiamycin-treated, virus-infected cells*

Virginiamycin			Virus	Labeling (min)	Chasing (min)	Radioactivity in RNA[b]						Ratio $^{14}C/^{3}H$ in rRNA
Type	Amt (μg/ml)	Min				>23S	23S	16S	10S	4S	<4S	
			−	5-13		0.24	3.32	3.27	2.37	9.57	3.04	0.346
			−		13-23	0.20	4.57	4.50	2.18	7.37	2.40	0.282
			+	5-13		0.31	2.78	2.80	2.26	7.47	5.14	0.454
			+		13-23	0.15	2.54	2.94	2.02	5.10	1.66	0.413
M	100	−3	+	5-13		0.58	1.21	1.42	1.27	3.64	1.76	0.176
			+		13-23	0.94	1.77	2.36	1.74	6.75	2.01	0.148
S	100	−3	+	5-13		0.37	1.37	1.41	1.14	4.59	1.68	0.170
			+		13-23	0.77	1.24	2.31	2.51	3.73	2.07	0.163
M + S	10	−3	+	5-13		0.53	1.72	1.99	1.98	4.95	1.88	0.137
			+		13-23	1.15	2.10	2.02	3.22	7.68	2.10	0.118
			−	15-23		0.14	3.94	4.98	1.65	11.32	1.94	0.350
			−		23-33	0.34	6.94	7.28	2.34	9.31	4.35	0.264
			+	15-23		0.03	1.03	1.21	1.52	8.90	2.13	0.593
			+		23-33	0.05	1.16	1.33	0.66	3.00	1.22	0.399
M	100	−4	+	15-23		0.16	0.88	0.95	0.58	2.90	1.01	0.219
			+		23-33	0.21	0.96	1.36	0.82	3.29	1.08	0.234
S	100	−4	+	15-23		0.41	0.62	0.77	0.82	3.16	1.32	0.172
			+		23-33	0.53	0.69	1.31	0.83	3.30	0.76	0.180
M + S	10	−4	+	15-23		0.43	0.88	1.28	0.90	2.26	1.04	0.147
			+		23-33	0.06	0.92	1.45	1.37	4.97	1.38	0.191
M	100	+13	+	17-27		0.20	0.90	1.04	1.08	4.31	1.52	0.212
			+		27-37	0.49	1.27	1.70	0.87	3.88	1.14	0.177
S	100	+13	+	17-27		0.07	0.65	0.75	0.63	4.62	0.96	0.230
			+		27-37	0.17	1.01	1.47	0.97	3.15	0.86	0.170
M + S	10	+13	+	17-27		0.32	1.12	1.46	1.02	4.04	1.12	0.163
			+		27-37	0.59	1.12	2.19	1.07	4.89	2.20	0.130

[a] Infection at time zero with 15 PFU of virus 2C per cell.
[b] Acid-insoluble counts per minute $\times 10^{-3}$ of L-[*methyl*-^{14}C]methionine.

After short pulses, the radioactivity and the hybridization tracings essentially overlapped; both showed a main peak in the 10*S* region (Fig. 4A). As the labeling period was prolonged, the heterogeneity of RNA hybridizing with 2C DNA increased; though the main hybridization peak was still found in the 10*S* region, the radioactivity tracing showed additional 16*S* and 23*S* components (Fig. 4B). This indicates that in virus-infected cells, formation of cellular and viral RNA simultaneously occurs; the former corresponds to 23*S* and 16*S* rRNA, and the latter corresponds to mRNA of different molecular weights. The RNA made in the presence of virginiamycin was even more heterogeneous: although the main component was still 10*S* RNA, the proportion of RNA of higher and lower sedimentation coefficients increased (Fig. 5A-C). Apparently, virginiamycin did not prevent the synthesis of virus-specific RNA, but rather interfered with its metabolism.

Strand identification experiments showed a preferential but not exclusive hybridization of the RNA labeled after infection with the heavy strand of 2C DNA. Virginiamycin prevented such asymmetrical transcription of the viral genome (Table 5).

DISCUSSION

It is well established that the accumulation of cellular nucleic acids is blocked after the attachment of T-even phages to *E. coli* (10). More precisely, formation of 23*S* and 16*S* rRNA (24), tRNA (24), and cellular mRNA (25, 27) was found to be halted in T4-infected *E. coli*. This pattern is quite specific for phages T2 and T4, however, since it does not occur in *E. coli* infected either with phage λ (33) or with φX174 (28, 31). Moreover, virus T4 produces unlike metabolic pictures in different enterobacteria: though RNA formation is completely blocked in *E. coli* from the very beginning of the viral cycle, it is only partly and progressively inhibited in *Shigella* (32). The latter schema is shared by 2C-infected *B. subtilis* (Tables 1 and 2). Actually, the synthesis of cellular RNA is not

TABLE 3. *Metabolism of methylated RNA in virus-infected, virginiamycin-treated cells*

Virginiamycins M + S		Virus[a] (PFU/cell)	Labeling (min)	Chasing (min)	Radioactivity in RNA[b]						Ratio $^{14}C/^{3}H$ in rRNA
Amt (μg/ml)	Min				>23S	23S	16S	10S	4S	<4S	
			1-6		1.06	2.87	2.82	2.93	20.04	8.56	1.134
			1-6	6-16	1.20	4.48	5.95	3.91	15.34	4.37	0.787
		10	1-6		0.46	1.78	1.99	1.44	17.25	5.35	0.648
		50			0.98	1.34	1.62	1.02	14.76	4.27	0.647
		200			0.34	1.10	1.52	1.08	10.58	2.56	0.745
		10	1-6	6-16	0.39	2.31	2.35	2.05	9.27	3.17	0.869
		50			0.67	2.30	2.32	2.81	7.88	5.01	0.788
		200			0.49	1.56	2.22	0.76	7.86	2.01	0.875
10	-3	10	1-6		1.10	2.99	2.88	1.89	8.90	3.29	0.250
		50			0.91	2.09	2.56	1.77	7.32	2.59	0.264
		200			0.49	1.09	1.59	1.14	5.09	1.53	0.343
10	-3	10	1-6	6-16	0.95	1.66	3.19	2.46	19.94	7.19	0.288
		50			0.59	0.84	2.41	0.99	11.92	3.17	0.471
		200			1.32	0.78	2.10	0.87	8.78	2.57	0.533
		10	12-17		0.61	1.06	1.10	2.58	11.20	4.23	1.210
		50			0.46	0.76	0.64	1.56	12.53	1.98	0.974
		200			1.50	0.64	0.61	3.12	9.80	3.53	1.097
		10	12-17	17-27	—	3.85	3.41	5.85	8.23	1.77	1.054
		50			—	1.37	2.06	1.15	5.15	1.32	0.805
		200			0.09	2.02	2.72	1.61	5.94	1.37	0.830
10	-3	10	12-17		0.58	2.10	2.19	1.87	8.56	3.52	0.274
		50			0.31	1.89	1.82	0.83	7.99	1.98	0.245
		200			0.23	0.41	0.58	0.31	5.69	4.57	0.492
10	-3	10	12-17	17-27	0.37	0.79	1.31	0.54	16.70	4.15	0.416
		50			0.30	0.49	1.36	0.75	10.67	3.77	0.435
		200			0.26	0.45	1.19	0.62	5.98	1.65	0.706
10	+13	10	12-17		0.78	2.81	3.38	1.78	16.67	3.50	0.408
		50			0.45	1.20	2.18	1.58	8.49	3.29	0.334
		200			0.26	1.18	1.92	1.25	8.63	3.21	0.371
10	+13	10	12-17	17-27	0.63	1.73	4.88	1.98	25.49	9.70	0.307
		50			0.32	1.11	3.60	0.98	13.77	3.15	0.327
		200			0.35	1.17	3.10	1.03	11.34	2.75	0.358

[a] Infection at time zero with different multiplicities of infection.
[b] Acid-insoluble counts per minute × 10^{-3} of L-[*methyl*-^{14}C]methionine.

TABLE 4. *Formation of virus-specific RNA in the presence of virginiamycin*

Virginiamycin		Labeling of RNA (min after infection)[a]	Hybridization (%) with viral DNA of RNA made in the presence of virginiamycin			
Amt (μg/ml)	Min before or after infection		None	M	S	M + S
50	-7	3-5	21.4	6.7	10.2	5.2
50	-7	3-18	17.1	14.0	15.2	7.6
100	-5	8-9	27.2	9.2	26.0	15.7
100	-5	8-18	17.5	9.2	13.8	9.3
100	-5	25-26	19.7	5.0	19.1	2.2
100	-5	25-35	20.1	13.7	22.5	5.2
50	+12	17-19	25.6	26.9	37.5	31.6
		17-32	31.1	33.6	31.8	28.1
50	+12	30-32	34.1	61.6	53.1	62.5
		30-45	12.8	2.5	9.1	3.6

[a] Infection at time zero with 30 PFU of CsCl-purified virus 2C per cell.

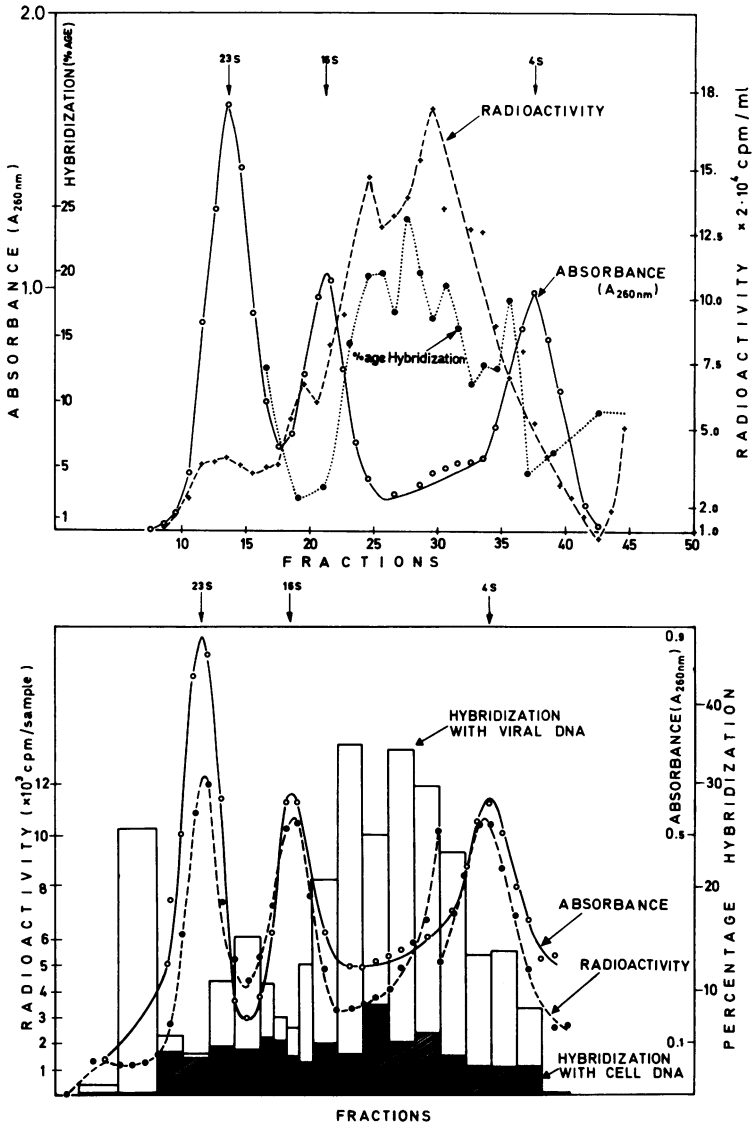

FIG. 4.

abolished even by very high multiplicities of virus 2C (Table 3).

When chloramphenicol is added before infection of *E. coli* with phage T4, the synthesis of different species of rRNA and tRNA continues throughout the viral cycle, whereas a late addition of this inhibitor is without effect (1, 23, 24, 32). Though data present here do not fit this schema, it would be misleading to overemphasize the difference between the chloramphenicol and the virginiamycin results. In reality, Tables 2, 3, and 4 indicate that formation of cellular RNA precursors increases in the presence of virginiamycin as it does in chloramphenicol-treated cells. What makes the difference is the fact that rRNA maturation is unaffected by chloramphenicol but drastically impaired by virginiamycin. Moreover, a possible derepression effect can be shown more easily in a fully repressed system such as that of T4 *E. coli* than in a partly inhibited system such as that of 2C-infected *B. subtilis*.

Inhibition of rRNA formation in 2C-infected *B. subtilis* was proportional to the multiplicity of infection (Table 3). A similar observation was made by others with T4-infected *Shigella* (32). On the other hand, in *E. coli*, T4 causes a repression of RNA synthesis that is independent

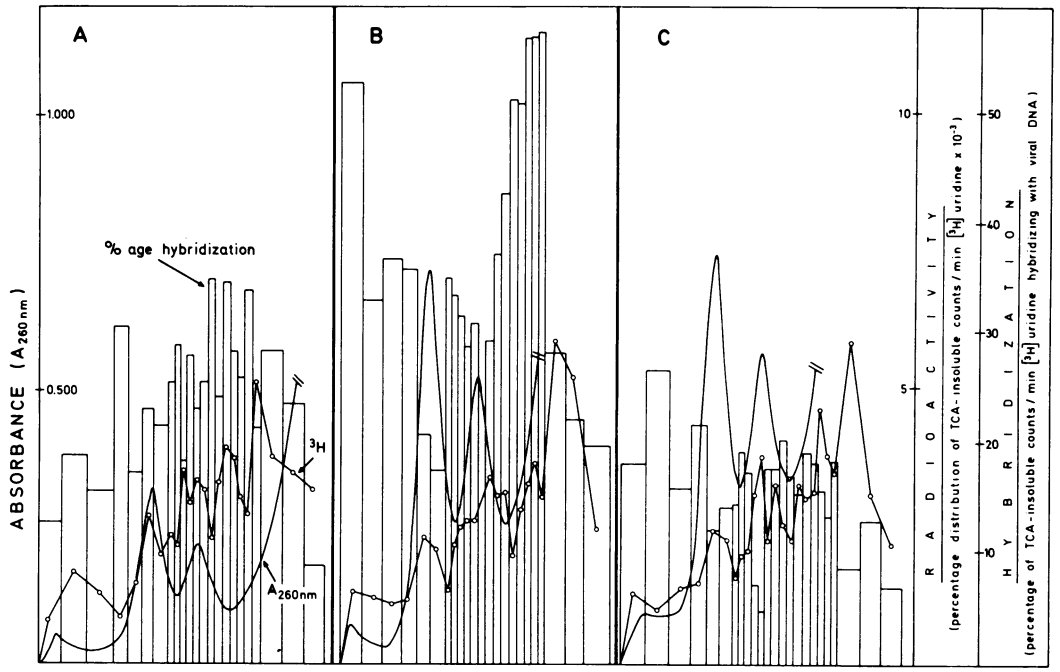

FIG. 5.

FIG. 4 AND 5. *Ultracentrifugal fractionation of phage-specific RNA. Cultures of A26, infected with 30 PFU of virus 2C per cell in the presence or absence of virginiamycin (50 μg either M or S and 5 μg of both components per ml) were labeled with either 1- or 15-min pulses, either during the eclipse phase (5 min) or during the maturation phase (25 min). RNA extracted from cell homogenates was fractionated by centrifugation in density gradients, and fractions were hybridized with viral DNA on membrane filters. (4A) 2C-infected cells labeled for 1 min in the absence of virginiamycin. (5A-C) 2C-infected cells, labeled for 1 min in the presence of virginiamycin M (A), S (B) and M + S (C). (4B) Virus-infected cells labeled for 15 min in the absence of virginiamycin.*

of the virus-to-cell ratio; a multiplicity effect can be shown, however, in the presence of chloramphenicol (32). Hence, inhibition of host RNA formation is proportional to the number of invading particles, provided duplication and transcription of the cell genome are not completely repressed. Such an extreme case occurs only in a few virus-cell systems and is probably due to some protein coded for by the virus, since it is prevented by inhibitors of protein synthesis. It must be mentioned in this respect that two kinds of RNA, sensitive and insensitive to chloramphenicol, have been described in T4-infected *E. coli* (18).

Unlike the well-known case of T2 *E. coli*, virus 2C does not produce a lysis from without in *B. subtilis* (cf. Table 3). Consequently, phage lysozyme cannot be responsible for this phenomenon as previously suggested.

Interference of virginiamycin with rRNA methylation and stability has been already shown in a previous publication (4). Similar observations were made by others for chloramphenicol (14) and amino acid starvation in relaxed mutants (22). Apparently, maturation and methylation of rRNA are late processes that

TABLE 5. *Hybridization of mRNA with separate strands of 2C DNA*

Virginiamycin[a]	mRNA[b]	Hybridization (%) with 2C DNA		
		Heavy strand	Light strand	Both
–	Early	42.6	12.3	62.6
+	Early	33.7	22.1	34.4
–	Late	31.4	14.4	67.1
+	Late	17.6	25.6	56.4

[a] Virginiamycin (50 μg of M + S per ml) added 10 min before the virus (30 PFU/cell).

[b] One-minute pulse of labeled uracil administered either 10 or 30 min after infection.

are somehow linked to the synthesis of ribosomal proteins and to the assembly of ribosomal particles. It is not surprising, therefore, that an inhibitor of ribosomal protein formation does interfere with rRNA methylation. Instead, more puzzling is the mechanism by which phage 2C prevents the virginiamycin-dependent inhibition of rRNA methylation. The simplest explanation is that the complex of viral DNA and polymerase competes with polysomes for fixation of virginiamycin. This view is supported by several findings. First, virginiamycin blocks 2C DNA formation at any moment of the viral cycle, i.e., when viral polymerase is already present (5). Second, this antibiotic inhibits cellular DNA formation in *Plectonema boryanum*, presumably through fixation to DNA-protein complexes.

An asymmetrical transcription of phage DNA has been reported already by several authors and found to depend on the structure of the viral genome (12, 15, 20, 30, 34). Moreover, a transcription of different portions of both DNA strands, occurring in opposite directions at different times of the replication cycle, has been shown for phages λ (cf. ref. 16) and T5 (17). Data reported in Table 5 can be interpreted along these lines, i.e., by assuming periodical shifts of the transcription process between the heavy and the light parental strands. Such a hypothesis is now being analyzed in our laboratory. In addition, our results indicate that asymmetrical transcription is due to some virus-dictated proteins, since this process is rendered symmetrical by virginiamycin.

ACKNOWLEDGMENTS

Preliminary experiments along these lines were carried out at the Department of Biochemistry, Albert Einstein College of Medicine, Bronx, N.Y. I am indebted to J. Marmur for hospitality and suggestions and to A. Fowler for invaluable help in hybridization techniques.

This investigation was supported by a grant of the Belgian National Science Foundation (FRSM-20024).

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