Asymmetric Transcription of Bacteriophage Mu-1

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The deoxyribonucleic acid (DNA) of bacteriophage Mu-1 can be separated into its complementary strands by poly(U,G) binding and equilibrium centrifugation. DNA-ribonucleic acid (RNA) hybridizations in liquid show that more than 98% of "early" phage-specific RNA and over 96% of "late" messenger species bind to the heavy [poly(U,G)-binding] strand of Mu-1 DNA. A small (1.45%) but significant amount of late RNA binds to the light strand. The significance of this RNA fraction is discussed in connection with the peculiar structure of denatured and reannealed Mu-1 DNA.

The temperate bacteriophage Mu-1 has the remarkable ability to lysogenize *Escherichia coli* by inserting its deoxyribonucleic acid (DNA) at random locations in the host chromosome (2, 11). In the lysogenic state, the phage DNA is inserted linearly into the *E. coli* chromosome (7). The lytic cycle of Mu-1 has a duration of 55 to 60 min. Synthesis of phage-specific ribonucleic acid (RNA) occurs in two phases, with both "early" and "late" RNA complementary to the same (heavy) strand of Mu-1 DNA.

MATERIALS AND METHODS

Bacteria and phage strains. Strain AT731 (from A. L. Taylor) is HfrC *metB proB lacY* (Mu-1) and carries a heat-inducible prophage in the *lacY* gene. Strain 7566 is *E. coli* W3110 lysogenized with a heat-inducible mutant of phage Mu-1 (*cts62* from M. Howe) at an unknown position on the bacterial chromosome. Strain W3110 *thy*⁻ (from J. Cairns) was also used as host for lytic growth of wild-type Mu-1.

Preparation of phage. Strain AT731 was grown at 32°C in LB broth (per liter: tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; pH 8.0); W3110 and its lysogenic derivative were grown in the same medium supplemented with thymine at 40 µg/ml. At a cell concentration of 2×10^8 to 3×10^8 /ml, the prophages were induced by vigorous agitation of the cultures for 30 min at 45°C. After induction, the cultures were grown at 37°C until visible lysis occurred. Wild-type Mu-1 was prepared by low-multiplicity infection under the conditions described by Martuscelli et al. (8). Lysates containing 10^{10} to 2×10^{10} plaque-forming units per ml were consistently obtained. The lysates were concentrated as suggested by Martuscelli et al. (7) or by differential centrifugation. The concentrated phage suspensions were then adjusted to a density of 1.496 g/cm³ with solid CsCl and centrifuged to equilibrium at 4°C. The phage bands were collected after 30 hr of centrifugation at 30,000 rev/min in a Spinco SW50 rotor. Phage particles purified in this way are stable for at least 2 weeks in the cold.

Preparation of complementary strands of Mu-1 DNA. Phage particles purified by two cycles of equi-

librium centrifugation were dialyzed for 12 to 18 hr against 1 mM ethylenediaminetetraacetic acid, pH 8.0, and treated with Sarkosyl NL97 (Geigy) in the presence of poly(U,G) (lot no. 2, Miles Laboratories) as described by Bøvre and Szybalski (1). The DNA-poly(U,G) mixture was adjusted to a density of 1.72 g/cm³ by the addition of a saturated solution of CsCl in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.2, and centrifuged for 60 hr at 10°C and 30,000 rev/min in a Spinco SW50L rotor. The pooled fractions were dialyzed for 12 to 18 hr at 4°C against three changes of $2 \times$ SSC (0.3 M NaCl-0.030 M sodium citrate, pH 7.4) and heated (self-annealed) for 3 hr at 66 to 67°C. In some cases the dialyzed fractions were adjusted to 0.3 N NaOH and incubated for 16 hr at 37°C to remove poly(U,G). The treated strands were then dialyzed and self-annealed as before. The alkali treatment resulted in some breakage of DNA strands as shown by electron microscopy. Complementarity of the separated strands was controlled by electron microscopy of equal amounts of the two separated strands annealed in mixture for 5 hr. Samples for electron microscopy were prepared by the method of Davis et al. (4).

Preparation of RNA and DNA-RNA hybridization. Labeled RNA was prepared from 20-ml cultures grown in a minimal salt medium (5) with succinate as carbon source and supplemented with 0.2% vitamin-free Casamino Acids (Difco) and 40 µg of thymine per ml. At a concentration of 2×10^8 cells per ml the prophage was induced, and ³H-uridine (Schwarz BioResearch) was added to 5.0 µCi/ml at different times after the start of induction. Incorporation was stopped after 2 min by pouring the cultures over 5 ml of frozen and crushed M9b medium (1) supplemented with 0.02 M Na₂Na and 200 µg of chloramphenicol per ml. For analysis of RNA produced during the lytic cycle after infection, cultures of W3110 were infected at 2×10^8 cells per ml with wild-type Mu-1 (5 phage particles per cell). Fifteen minutes before infection these cultures were supplemented with 0.001 M CaCl₂ and 0.0025 M MgSO₄. After centrifugation, the bacterial pellet was frozen at -80°C for a few minutes, and the RNA was extracted as described by Okamoto et al. (9). The aqueous phase from the last of four

phenol extractions was adjusted to 0.3 M sodium acetate and precipitated with ethanol. The precipitate was resuspended in 0.5 ml of 0.02 M Tris-hydrochloride (pH 7.2) and 0.005 M MgSO₄ and incubated for 60 min at 37 C with 25 µg of ribonuclease-free deoxy-ribonuclease (Worthington) per ml and thereafter for 2 hr with 50 µg of self-digested Pronase (Calbiochem) per ml. The RNA was extracted as before and resuspended in 2 × SSC saturated with phenol at 4 C. Hybridization with separated strands was performed for 5 hr at 66 to 67 C (1). Changes in the amount of phage-specific RNA in the cells are calculated from the percentages of label added to the hybridization mixture that bind to separated strands. Early RNA is RNA extracted from induced or infected cells up until 20 min after the beginning of the lytic cycle. Late RNA corresponds to 30- to 55-min RNA (unpublished hybridization-competition experiments).

RESULTS AND DISCUSSION

The native DNA of bacteriophage Mu-1 has a base composition and a density in neutral CsCl like that of *E. coli* K-12 (7). After denaturation, the phage DNA behaves as a single species having a density of 1.710 g/cm³ in neutral CsCl. In alkaline CsSO₄, denatured Mu-1 DNA sediments as two slightly separated fractions (7). Equilibrium centrifugation in CsCl in the presence of poly(U,G) permits separation of the complementary strands of Mu-1 DNA (Fig. 1). Electron microscopy examination of the purified and self-annealed strands shows intact strands, a small population of fragments, and a small amount of double-stranded DNA as expected from the partial overlapping of the fractionation pattern.

FIG. 1. Fractionation of the complementary strands of Mu-1 DNA. Four absorbancy units at 260 nm of dialyzed Mu-1 phage (about 200 µg of DNA) were treated and centrifuged as described in Materials and Methods. Fractions of five drops each were collected from the bottom of the tubes. Samples of 20 µliters were diluted in 300 µliters of 1 × SSC and used for measurement of absorbancy at 260 nm. The arrows indicate positions of the peaks of the heavy and light strands of T7 DNA, prepared and centrifuged at the same time under identical conditions.

TABLE 1. Hybridization pattern of Mu-1-specific RNA^a

Strand ^b	Condition	Lysogenic not induced	Time (min) after induction of the prophage					
			5-7	10-12	20-22	30-32	40-42	50-52
Heavy	Counts/min bound to filter	45 ± 9 (4)	552 ± 53 (2)	305 ± 91 (3)	396 ± 150 (3)	14900 ± 900 (2)	27944 ± 1270 (3)	14000
	Input (counts/min) % Hybridized	39870 ± 1080 0.114	22000 ± 3000 2.50	23700 ± 2200 1.28	15500 ± 505 2.55	43750 ± 1760 34.0	93000 ± 6820 30.1	41500 30.0
Light	Counts/min bound to filter	31 ± 9 (3)	10 ± 10 (2)	6 ± 5 (3)	5.3 ± 2.8 (3)	645 ± 56 (2)	598 ± 73 (3)	600
	Input (counts/min) % Hybridized	39870 ± 1080 0.078	22000 ± 3000 0.046	23700 ± 2200 0.025	15500 ± 505 0.034	43750 ± 1760 1.48	93000 ± 6820 0.65	41500 1.45

^a Hybridizations with RNA labeled for 2 min were performed as described in Materials and Methods at saturating DNA concentrations. Blanks (35-45 counts/min) with 0.4 µg of separated strands of T7 were subtracted from all values. Figures indicate mean values ± standard error; figures in parentheses, number of different RNA preparations analyzed.

^b 0.2 µg of DNA per assay.

The number of small pieces is notably increased in preparations treated with alkali to remove poly(U,G). A mixture of equal amounts of each strand annealed for 5 hr at a concentration of 5 μ g/ml gives rise to double-stranded DNA, thus confirming complementarity of the strands.

The lytic cycle of Mu-1 is completed in 55 to 60 min, both after induction of a temperature-sensitive prophage and after infection of sensitive cells at 38 C. Only a small amount of labeled RNA from lysogenic, noninduced cells binds to Mu-1 DNA (Table 1). We are studying this observation further in connection with the capacity of Mu-1 as a transducing phage. The synthesis of phage-specific RNA occurs in two phases, after both induction and infection (Table 1). During the first phase (5 to 22 min), the amount of Mu-1-specific (early) RNA increases 10- to 15-fold with respect to the levels in the uninduced or uninfected cells. This early RNA binds exclusively to the heavy strand and still represents less than 5% of the pulse-labeled RNA in the cells. The second increase in the synthesis of phage-specific messenger occurs between 20 and 30 min after induction or infection; 30 min after the beginning of the lytic cycle, as much as 30% of the total mRNA is phage specific. Even shortly before lysis there is no decrease in the rate of synthesis of Mu-1-specific RNA. The second increase in the synthesis of Mu-1 RNA (late RNA) measures a 10-fold increment over the amount of early RNA complementary to the heavy strand. The percentage of RNA binding to the light strand increases to the same extent with respect to uninduced levels but never represents more than 4% of the total late RNA. It has been shown (*unpublished results*, with H. Delius) that denaturation and reannealing of Mu-1 DNA (13.4 μ m) extracted from phage particles results in peculiar structures consisting of 11.64 μ m of completely renatured DNA, followed by a "bubble" (1.11 μ m), and separated from the split end of the renatured molecule (0.45 to 0.59 μ m long) by a small (0.59 μ m) segment of completely renatured DNA. The bubble has been shown to correspond to an inversion (Ming-ta Hsu, *personal communication*) and to contain a segment (about 0.34 μ m long) of self-complementary DNA (*unpublished results*, with H. Delius). The presence of this inversion with the inverted duplication could explain the binding of late RNA to the light strand. Further experiments should show whether this species of late RNA is transcribed from only one or from both strands, but the present results already suggest that this segment of Mu-1 DNA corresponds to late phage functions.

The results show that, in temperate bacteriophage Mu-1, unlike the lambdoid phages (3, 6, 10), both early and most late RNAs are transcribed from the same (heavy) strand. Experiments are also being performed to decide whether this transcription pattern is similar to that of T7 (9) or whether several equally strong promoters for the *E. coli* polymerase are scattered along the DNA molecule of Mu-1. We are also continuing the use of this system to gain further insight into how Mu-1 DNA is integrated into the host chromosome.

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