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Class of φX174 Mutants Relatively Deficient in Synthesis of Viral RNA

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Nonpermissive cells infected with φX174 gene *D* amber mutants synthesized some sixfold less viral RNA than permissive cells. The decrease was unaffected by increasing the multiplicity of infection and was a consequence of an overall decrease in all viral RNA species. It is suggested that the gene *D* product may function in replicative form DNA unwinding to expose the template for transcription.

The bacteriophage φX174 gene *D* product, a protein of 14,500 molecular weight, is made in largest molar amount of any φX gene product in infected cells (2, 3). The precise function for this protein is not known, however; mutants in gene *D* do not form progeny single-stranded DNA, but do make a normal amount of double-stranded, replicative-form (RF) DNA (6, 8). The present study examines the synthesis of φX mRNA in cells infected with gene *D* amber mutants.

Escherichia coli HF4704 (7) was the nonpermissive host for viral amber mutants, and *E. coli* HF4714 was the permissive host. Stocks of the gene *D* mutants used (*am*10 and *am*42), obtained from R. M. Benbow, had a reversion frequency of 3×10^{-5} and were prepared as described previously (2). Cells were grown to a density of 5×10^8/ml in 20 ml of TPA medium (13) containing one-tenth the normal phosphate concentration and supplemented with a mixture of 20 natural *l*-amino acids (2.7 g/liter) (Nutritional Biochemicals Corp., Cleveland, Ohio) and 10 μg of thymine per ml. Virus was then added at the multiplicities of infection described below. Twelve minutes after infection, cultures were pulsed with [^{32}P]phosphoric acid (carrier free) at 100 μCi/ml for 5 min. RNA was isolated by phenol extraction, and the proportion of φX mRNA was determined by hybridizing to denatured φX RF DNA. RNA samples were fractionated by polyacrylamide gel electrophoresis and sedimentation in dimethyl sulfoxide gradients.

Table 1 shows the proportion of φX mRNA in the total labeled RNA isolated from permissive and nonpermissive cells infected with gene *D* mutants. Values obtained with φX *am*3, a lysis-deficient gene *E* mutant (5), are included for comparison. The amount of φX mRNA synthesized in nonpermissive cells was reduced sixfold as compared with the values obtained from permissive cells. Increasing the multiplicity of infection by 10-fold had no influence upon this effect. This decrease was not shown with RNA from cells infected with the gene *E* mutant, where values with nonpermissive cells were in fact somewhat higher due to lysis inhibition. (Likewise, no decrease was observed in the amount of φX-specific RNA obtained from nonpermissive cells infected with *am*16, a mutant in gene *B*, which, like gene *D*, is required for single-stranded DNA synthesis.)

In Fig. 1, the gel profiles of φX mRNA in permissive and nonpermissive cells infected with *am*42 at a multiplicity of 4 show no obvious evidence for a specific decrease in any of the discrete φX mRNAs. This also was true for *am*10 infected cells (data not shown). On denaturing dimethyl sulfoxide gradients (Fig. 2), the pattern of distribution of RNA counts hybridizable to φX RF DNA was similar with RNA obtained from permissive and nonpermissive cells. This result indicates the absence of internal nicks in RNA made under nonpermissive conditions.

The decrease in the amount of φX mRNA in cells infected with gene *D* mutants is reflected in a reduction of the amount of virus-specific proteins (2). A decrease in the amount of virus mRNA in cells infected with gene *A* mutants of the related phage S13 has been observed (11). However, in this case, the amount of S13 mRNA was increased by raising the multiplicity of infection, a result attributed to an increase in the amount of RF template, as gene *A* mutants are unable to replicate RF DNA (14).

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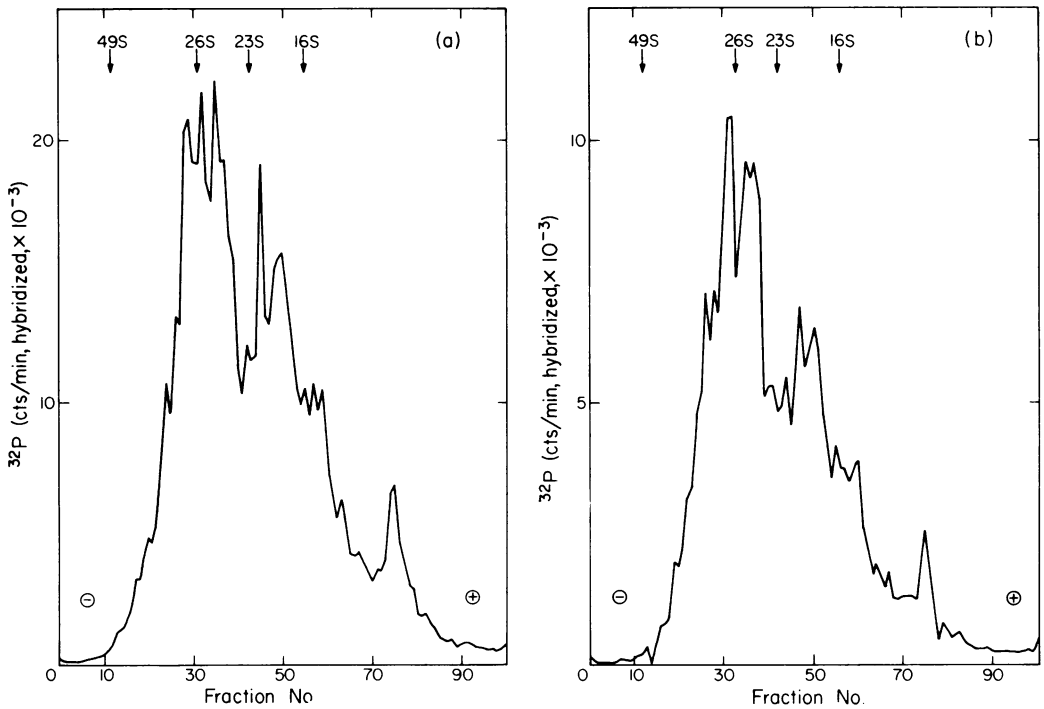

FIG. 1. *Gel electrophoresis of φX mRNA. RNA samples (25 to 50 μg) were coelectrophoresed with ^{3}H-labeled E. coli rRNA and ^{3}H-labeled Sindbis-specific RNA on 2% polyacrylamide, 0.4% agarose gels at 6 mA/gel for 4 h at 4 C. Gels were cut into 1-mm segments, and slices were agitated in a horizontal shaker in vials containing 0.4 ml of 50% (vol/vol) formamide-6 × SSC (pH 7.0) at 37 C for 48 h. After elution, a 20-μliter sample was taken for assay of radioactivity, then a blank filter, followed by an RF DNA filter was added. Vials were incubated at 37 C for 30 h, and filters were processed as described in Table 1. (a) RNA from permissive cells infected with am42. (b) RNA from nonpermissive cells infected with am42. Arrows indicate the positions of 16S and 23S E. coli rRNA's and 26S and 49S Sindbis-specific RNAs.*

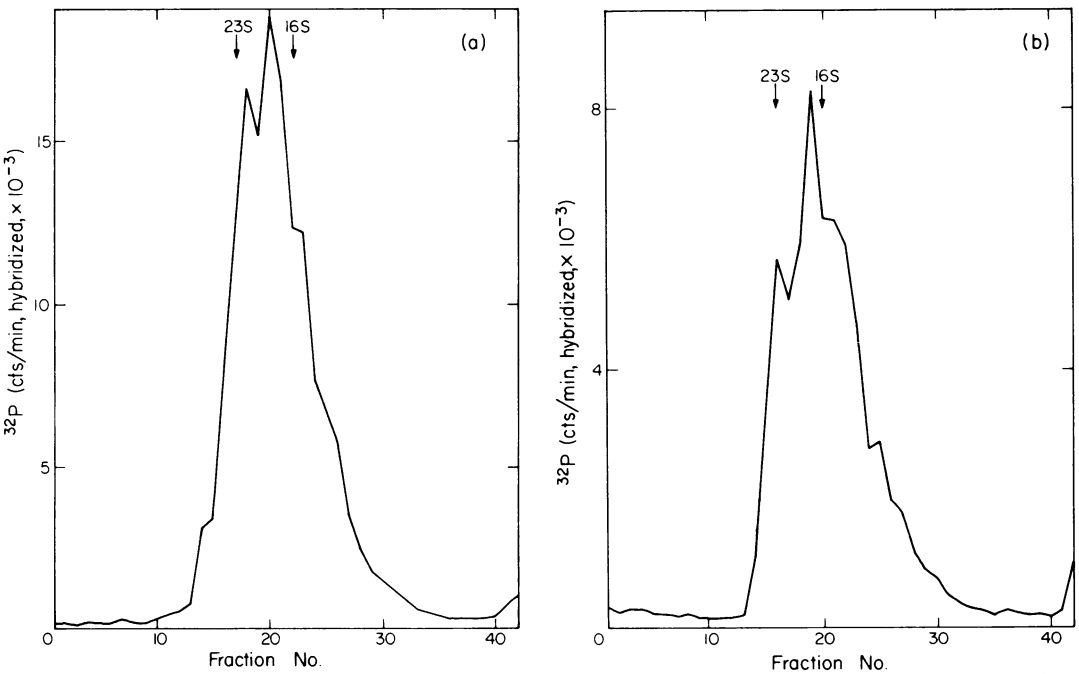

FIG. 2. *Sedimentation of φX mRNA on dimethyl sulfoxide gradients. RNA samples were cosedimented with ^{3}H-labeled E. coli rRNA in deuterated dimethyl sulfoxide gradients (12). Gradients were centrifuged at 64,000 rpm for 11 to 12 h at 27 C in a Spinco SW65 rotor. After counting a 10-μliter sample of each collected fraction, φX mRNA was located by hybridization to RF DNA in 40% (vol/vol) dimethyl sulfoxide-2 × SSC (pH 7.0) at 37 C for 50 h. Arrows indicate the position of 16S and 23S E. coli rRNA's. Sedimentation is from right to left. (a) RNA from permissive cells infected with am42. (b) RNA from nonpermissive cells infected with am42.*

TABLE 1. *Proportion of φX mRNA in permissive and non-permissive cells*[a]

Phage strains	Host strains	
	HF4704 (Su$^+$)	HF4714 (Su$^-$)
φX *am*10 (*D*)	1.62 ± 0.6 (4)	8.32 ± 1.1 (4)
	1.37 ± 0.3 (40)	7.63 ± 0.8 (40)
φX *am*42 (*D*)	1.17 ± 0.8 (4)	7.16 ± 0.5 (4)
	1.03 ± 0.6 (40)	7.70 ± 0.9 (40)
φX *am*3 (*E*)	14.55 ± 2.1 (4)	9.15 ± 0.7 (4)
	16.32 ± 2.7 (40)	10.27 ± 1.4 (40)

[a] The amount of φX hybridizable RNA is expressed as a percentage of total labeled RNA. Each value is the mean of six separate estimations with errors expressed as extreme fluctuations from the mean. Figures in parentheses show the multiplicity of infection. Radioactive pulses were terminated by addition of a sodium azide (0.5 M) and potassium cyanide (0.03 M) mixture to final concentrations of 0.05 M and 0.003 M, respectively. Cultures were immediately frozen by immersion in a dry ice-methanol bath. The cell pellets were suspended in 6 ml of Tris-NaCl-EDTA buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, pH 7.5) containing 0.2 mg of lysozyme per ml. These suspensions were maintained at 22 C for 3 min, then sodium dodecyl sulfate was added to 0.5%. The lysates were extracted twice at 22 C with phenol equilibrated with Tris-NaCl-EDTA plus 0.5% sodium dodecyl sulfate. RNA was precipitated twice from 10 ml of Tris-NaCl-EDTA with 3 volumes of 95% ethanol at −20 C. The precipitates were then suspended in 1 ml of 0.1 M sodium acetate, 2.5 mM magnesium sulfate (pH 5.0) containing 25 μg of deoxyribonuclease I (electrophoretically purified; Worthington Biochemicals Corp., Freehold, N.J.). After incubation at 22 C for 30 min, the samples were extracted with phenol, the RNA was reprecipitated twice, and samples were stored in ethanol at −20 C. More than 95% of the product was soluble in trichloroacetic acid after treatment with 0.3 N KOH (16 h, 37 C) or with 20 μg of RNAse A per ml (1 h in 0.1 × SSC). RNA samples (0.5 to 5 μg of total RNA) in 0.4 ml of 6 × SSC were hybridized to 10-μg lots of denatured, DNA (1 to 2 μg was sufficient to hybridize all the φX mRNA) on 11-mm nitrocellulose filters at 66 C for 30 h in the presence of blank control filters. After incubation, filters were washed twice in 10 ml of 2 × SSC, and then treated with 1 ml of pancreatic ribonuclease (25 μg of 2 × SSC per ml (pH 5.0), preheated to 84 C for 10 min to eliminate DNase) for 40 min at 22 C. After two washes with 2 × SSC, the dried filters were counted in a toluene-based scintillation fluid. Counts on blank filters were subtracted from values of the appropriate RF filter.

In terms of molecular weight, and molar amount synthesized, the φX gene *D* product seems analogous to the gene 5 product of the filamentous phages, e.g., (f1). The gene 5 product has been characterized; it binds tightly to single-stranded DNA and denatures double-stranded DNA at physiological temperatures (1, 9, 10). The reduction in the amount of φX mRNA synthesized in infections with gene *D* mutants might be ascribed to a role for the gene product in RF unwinding, so as to expose the complementary DNA strand which is the template for RNA synthesis (4).

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