Escherichia coli Mutant Which Restricts T7 Bacteriophage Has an Altered RNA Polymerase

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We have previously described an *Escherichia coli* K-12 mutant, Y49, which restricts the growth of bacteriophage T7 and causes the accumulation of short DNA molecules and head-related particles during infection. We now show that the basis for these effects is the inability of the T7 gene 2 product to inactivate the Y49 RNA polymerase during infection, similar to what has been shown by DeWyngaert and Hinkle (J. Biol. Chem. 254:11247-11253, 1979) for the BR3 and *tsnB* strains of *E. coli*.

The bacteriophage T7 gene 2 product, gp2 or I-protein, is essential for productive infection by T7 (17). Its interaction with *Escherichia coli* RNA polymerase results in a specific inhibition of the host enzyme (8-10). This role of gp2 in the early-to-late transcriptional switch in T7 phage development has been established (9).

On the other hand, T7 gene 2 protein has been implicated in T7 DNA replication, particularly concatemer formation (3, 19). However, in abortive infection of *E. coli* with T7 gene 2 mutant phage, concatemeric T7 DNA molecules are formed, although they are not processed correctly and accumulate as pieces approximately 30% shorter than unit-size T7 DNA (15). At the same time, phage head-related particles also accumulate, and no infectious particles are produced (15). The addition of rifampin to a T7 2⁻-infected culture at the time of the early-to-late switch can rescue this abortive infection (15). Furthermore, in an in vitro T7 DNA packaging system which requires the presence of gp2, it has been shown that rifampin can complement an extract from T7 2⁻-infected cells (13).

During T7⁺ infection of the *E. coli* mutant Y49, short T7 DNA molecules, cleaved from concatemers, and head-related particles concomitantly accumulate with a poor production of progeny phage (21, 22), analogous to the observation with T7 2⁻-infected wild-type host cells described above. This resemblance suggested that Y49 RNA polymerase might be resistant to inhibition by the T7 gp2.

The addition of rifampin 10 min after infection did rescue abortive infection of Y49 cells, resulting in normal processing of concatemers and a yield of progeny phage equivalent to that of a wild-type host infection. In addition, no inhibi-

tion of *E. coli* RNA polymerase was observed throughout the course of T7⁺ infection of *E. coli* Y49.

To study directly the effect of gp2 on Y49 RNA polymerase, both the wild-type D10 (20) and mutant Y49 RNA polymerase holoenzymes were purified from uninfected cells (2). The wild-type enzyme was purified to a specific activity of 388 U/mg, and the Y49 enzyme was purified to 357 U/mg. Both were judged to be approximately 85% sigma-saturated. There was no discernible difference in the mobility of any of the subunits when the two enzymes were electrophoresed in parallel through polyacrylamide gels containing sodium dodecyl sulfate (12). Agarose-acrylamide gel analysis of the RNA transcripts synthesized in vitro by Y49 RNA polymerase with T7 DNA as a template revealed a predominant species of 2.5×10^6 daltons, the expected size of the major T7 early transcript (14).

The T7 gene 2 protein was purified from T7⁺-infected *E. coli* D10 by published procedures (5, 10, 13). The purified protein migrated as a single band when electrophoresed through a polyacrylamide gel (16) containing sodium dodecyl sulfate and 6 M urea. It migrated with an apparent molecular weight of 7,000, in excellent agreement with the value of 7,150 predicted from the DNA sequence (7).

The alteration in the Y49 RNA polymerase became evident when purified RNA polymerase activity was assayed in the presence of increasing amounts of purified gp2 (Fig. 1). The wild-type D10 holoenzyme was inhibited by gp2 as expected, but the Y49 enzyme was not. No inhibition of Y49 RNA polymerase was observed even at more than twice the concentration of gp2 required to fully inhibit the wild-type enzyme.

We have previously demonstrated that gp2

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FIG. 1. Activity of purified *E. coli* D10 (20) and Y49 RNA polymerases in the presence of purified T7 gp2. RNA synthesis was carried out as described (9). Each reaction contained 1 μ g of purified *E. coli* D10 or Y49 RNA polymerase holoenzyme, 3 μ g of T7 DNA, and the indicated amount of purified gp2 in a final volume of 125 μ l. Enzyme and gp2 were mixed and incubated before the addition of DNA and the nucleoside triphosphates. Activity was expressed as the percentage of RNA polymerase activity relative to the reactions which contained no gp2. Symbols: ●, *E. coli* D10 RNA polymerase activity; ○, *E. coli* Y49 RNA polymerase activity.

acts by binding directly to the RNA polymerase holoenzyme and preventing the enzyme from binding to the DNA template (10). The observed resistance of the Y49 RNA polymerase to inhibition by gp2 was verified by assaying the retention on nitrocellulose filters of the RNA polymerase-T7 DNA binary complex (Fig. 2). The presence of gp2 in the reaction did not affect the binding of Y49 RNA polymerase to T7 DNA (Fig. 2), consistent with the finding that RNA synthesis catalyzed by the Y49 enzyme was unaffected by the addition of gp2 (Fig. 1).

Although gp2 was not able to inhibit Y49 RNA polymerase, the question remained as to whether or not gp2 could bind to the altered enzyme. To answer this question, purified RNA polymerase and gp2 were mixed and centrifuged through glycerol gradients. Gradient fractions were assayed for both RNA polymerase and the inhibitory activity of gp2 (Fig. 3). When the wild-type enzyme and gp2 were mixed and centrifuged (Fig. 3b), some of the gp2 bound to the enzyme and therefore cosedimented with it. This result-

ed in an inhibition of the wild-type enzyme (compared to Fig. 3a) and a reduction in the amount of gp2 found in the upper portion of the gradient (compared to Fig. 3a). However, when the Y49 RNA polymerase was mixed with gp2 and centrifuged (Fig. 3e), all of the inhibitor activity was recovered in the upper portion of the gradient while the uninhibited enzyme activity was in the lower portion.

The results presented in this paper provide direct evidence that the *E. coli* K-12 mutant Y49 possesses an altered RNA polymerase which is resistant to inhibition by the gp2 of T7 phage. The gp2 resistance of strain Y49 RNA polymerase is due to its inability to bind gp2, although the enzyme retains normal functional activity and is sensitive to inhibition by rifampin. These

FIG. 2. Effect of gp2 on the binding of *E. coli* D10 or Y49 RNA polymerase to T7 DNA. The binding of RNA polymerase to T7 DNA was measured by the retention of ^3H -labeled T7 DNA on nitrocellulose filters as described (11). Reactions contained 1 μ g of purified D10 or Y49 RNA polymerase, 5 μ g of ^3H -labeled T7 DNA, and the indicated amounts of purified gp2. Enzyme and gp2 were mixed and incubated before the addition of DNA. Retention of T7 DNA in the absence of RNA polymerase was 5% of the input (29,000 cpm), and this value was subtracted from all points. Values of 100% (no gp2 present) for the D10 and Y49 enzymes represented retention of approximately 85% of the input counts per minute. No binding above background was observed in reactions containing T7 DNA and 5 μ l of gp2 but no RNA polymerase. Symbols: ●, Binding of D10 RNA polymerase; ○, binding of Y49 RNA polymerase.

FIG. 3. Glycerol gradient sedimentation analysis of the interaction between gp2 and D10 or Y49 RNA polymerase holoenzyme. Samples layered on the gradients contained: 30 μ g of purified D10 (A) or Y49 (D) RNA polymerase; 350 ng of gp2 and 30 mg of D10 (B) or Y49 (E) RNA polymerase; 350 ng of gp2 alone (C) and (F). Samples were centrifuged through 4.6-ml 10 to 30% glycerol gradients containing 0.15 M KCl (9). Centrifugation was in an SW50.1 rotor for 8 h at 50,000 rpm. Fractions 1 through 10 from (A), (B), (D), and (E) were assayed for RNA polymerase activity as described (9); reactions contained a 30- μ l sample from the gradient fraction and 2 μ g of T7 DNA. Fractions 11 through 20 from (B), (C), (E), and (F) were assayed for the inhibitor activity of gp2 (9); reactions contained a 50- μ l sample from the gradient fraction, 0.2 mg of D10 RNA polymerase holoenzyme, and 2 μ g of T7 DNA. Activity of gp2 was expressed as the percentage of inhibition of RNA synthesis relative to reactions containing an equivalent amount of RNA polymerase but no gradient fraction sample. Symbols: $\circ$, RNA polymerase activity; $\bullet$, inhibitor activity of gp2.

results are in good agreement with those of DeWyngeart and Hinkle (5), who found that the RNA polymerases of two independently isolated *E. coli* B mutants, BR3 (18) and *tsnB* (4), are both resistant to gp2 inhibition.

Y49, like BR3, was isolated by virtue of its inability to plate T7 0.7⁻ (kinase) phage, whereas *tsnB* does not even plate T7⁺. Although all three of these host mutants synthesize an RNA polymerase resistant to inhibition by the T7 gene 2 protein, DeWyngeart and Hinkle have noted that the *tsnB* mutation is greater than 90% cotransducible with the *rifA* locus of *rpoB* (β subunit) (6). P1 transductional analysis of the mutation in Y49 shows that it is only 67% cotransducible with the *rifA1* locus of AJ7 (1), indicating that the Y49 mutation lies in *rpoC* (β' subunit). Preliminary results of mixed subunit reconstitution experiments support the genetic data showing that it is the β' subunit of Y49 RNA polymerase that is altered.

Yamada et al. showed that the primary defect in T7 morphogenesis in the Y49 host is at a step

very late in the DNA processing and packaging pathway (21, 22). The fact that the addition of rifampin can rescue the abortive T7 infection of Y49 cells, as well as T7 2⁻ infection of a wild-type host, argues that uninhibited host RNA polymerase is responsible for the perturbation of the T7 DNA packaging processes. The observations that gp2 is a necessary component of an in vitro T7 DNA packaging system and that rifampin can effectively substitute for gp2 (13) support the notion that the essential function of gp2 is to allow T7 phage packaging to proceed unperturbed by the host RNA polymerase.

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