***Escherichia coli* Mutations That Prevent the Action of the T4 *unf/alc* Protein Map in an RNA Polymerase Gene**

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ABSTRACT

Bacteriophage T4 has the substituted base hydroxymethylcytosine in its DNA and presumably shuts off host transcription by specifically blocking transcription of cytosine-containing DNA. When T4 incorporates cytosine into its own DNA, the shutoff mechanism is directed back at T4, blocking its late gene expression and phage production. Mutations which permit T4 multiplication with cytosine DNA should be in genes required for host shutoff. The only such mutations characterized thus far have been in the phage *unf/alc* gene. The product of this gene is also required for the unfolding of the host nucleoid after infection, hence its dual name *unf/alc*. As part of our investigation of the mechanism of action of *unf/alc*, we have isolated *Escherichia coli* mutants which propagate cytosine T4 even if the phage are genotypically *alc*⁺. These same *E. coli* mutants are delayed in the T4-induced unfolding of their nucleoid, lending strong support to the conclusion that blocking transcription and unfolding the host nucleoid are but different manifestations of the same activity. We have mapped two of the mutations, called *paf* mutations for prevent *alc* function. They both map at about 90 min, probably in the *rpoB* gene encoding a subunit of RNA polymerase. From the behavior of *Paf* mutants, we hypothesize that the *unf/alc* gene product of T4 interacts somehow with the host RNA polymerase to block transcription of cytosine DNA and unfold the host nucleoid.

THE discovery that T even coliphages, including T4 and its relatives, shut off *Escherichia coli* gene expression after infection (MONOD and WOLLMAN 1947) was fundamental to many developments in molecular biology, including the discovery of mRNA and the elucidation of the function of ribosomes. Nevertheless, the molecular mechanisms by which the shutoff is achieved have never been understood, making this one of the longest standing mysteries in gene regulation in *E. coli*.

Presumably, T4 can block host transcription by specifically blocking transcription from cytosine DNA since its own DNA has the substituted base, glucosylated hydroxymethylcytosine which makes its own DNA distinguishable from host DNA. In support of this rationale, T4 with the right combination of mutations will replicate its DNA to contain cytosine but this DNA is not transcribed (KUTTER *et al.* 1975; WU and GEIDUSCHEK 1975) unless the T4 also have an *alc* mutation for allows lates on cytosine DNA (SNYDER, GOLD and KUTTER 1976; TIGGES, BURSCH and SNUSTAD 1977). As predicted, *alc* mutations also affect the shutoff of host transcription (SIROTKIN, WEI and SNYDER 1977; TIGGES, BURSCH and SNUSTAD 1977) although the details remain obscure. The *alc* mutations are now known to inactivate a 19-kD protein (HERMAN, HAAS and SNUSTAD 1984; KUTTER, DRIVDAHL and RAND 1984) which is encoded by the open reading frame immediately counterclockwise of

gene 63 (KUTTER, DRIVDAHL and RAND 1984; SNYDER and JORISSEN 1986). The gene has come to be called *alc* even though the normal product of the gene blocks transcription from cytosine DNA. The name *Alc* mutant is generally given to the phage which can multiply with cytosine DNA because they have an *alc* mutation and the other mutations required to replicate their DNA with cytosine. The *alc* gene product is also responsible for the "unfolding" of the host nucleoid after infection (TUTAS, WEHNER and KOERNER 1974; SNUSTAD *et al.* 1976; SIROTKIN, WEI and SNYDER 1977; TIGGES, BURSCH and SNUSTAD 1977; SNYDER and JORISSEN 1986) and the gene is sometimes given the dual name, *unf/alc*.

It is not clear how the *unf/alc* gene product acts to block transcription and unfold the host nucleoid. It seems likely that the *unf/alc* protein binds to RNA polymerase, DNA, or both. We once proposed (SIROTKIN, WEI and SNYDER 1977) that the *unf/alc* gene product bound to RNA polymerase and was probably the 15-kD RNA polymerase binding polypeptide first described by STEVENS (1972). We now know this conclusion was in error since the gene for the 15-kD polypeptide, *rpbA*, has been identified and is almost one-half way around the genome next to gene 45 (WILLIAMS *et al.* 1987; HSU *et al.* 1987). Recent biochemical evidence indicates that the *unf/alc* gene product binds to DNA (SNUSTAD, HASS and OPPENHEIMER 1986).

In this paper, we report the isolation of *E. coli* mutants in which the function of the T4 bacteriophage *unfI* gene product is substantially impaired. We have named the responsible *E. coli* mutations *paf* mutations because they prevent *alc* function. The *paf* mutations map close to, and probably in, the *rpoB* gene encoding a subunit of RNA polymerase, suggesting that the *unfI* gene product must interact with the host RNA polymerase to block transcription and unfold the host nucleoid.

MATERIALS AND METHODS

The bacterial strains and their relevant characteristics are shown in Table 1. The Hfr strains used for mapping were first made restrictionless because the *HsdR*⁺ phenotype is difficult to distinguish from the *Paf*⁺ phenotype, since both prevent the multiplication of cytosine T4 without an *alc* mutation. The *hsdR2* marker was transduced from *E. coli* LCK-8 selecting the tetracycline resistance of the closely linked *TN10* transposon and its presence established by cross-streaking with an *Alc* mutant with cytosine DNA. The restrictionless and suppressorless derivative of AB468, ADS-1, was constructed by introducing the *hsdR2* allele as above and crossing out the *supE* allele by conjugation with a *sup*⁰ Hfr, selecting *His*⁺. The *recA1* allele was introduced into ADS-1 by crossing with Hfr KL16-99, selecting *Pro*⁺, and identifying the *recA1* allele by sensitivity to ultraviolet light. Other auxotrophic markers were introduced by successive transductions or Hfr crosses, selecting one prototrophy and then screening for the other marker.

The *paf*-32 mutation was crossed onto the F' factor, F110, by crossing the F110 containing strain, KL727, with a derivative of the *Rec*⁺ strain, ADS-1, having the *paf*-32 mutation, a closely linked, dominant, rifampin resistance mutation and an *argECBH* mutation. About 10% of the *Arg*⁺ recombinants were killed by M13 so presumably had received the entire F' factor. One of the males was crossed with ADS-2 with the *recA1* allele. About 10% of the *Pur*⁺ apparent recombinants were rifampin resistant and all eight of these tested were phenotypically *Paf* so in these the rifampin resistance marker and the closely linked *paf* mutation had been crossed onto the F' factor in the recombination proficient donor.

For experiments requiring that T4 incorporate cytosine into its DNA, we used the T4 multiple mutant amE51, nd28, rH23 described previously (SNYDER, GOLD and KUTTER 1976) and referred to as T4EDD in the text. Because of the amber mutation, E51, in gene 56, the T4EDD phage will have glucosylated hydroxymethylcytosine DNA when propagated on amber suppressor *E. coli* but incorporate cytosine after it infects a nonsuppressor strain. The nd28 and rH23 mutations inactivate the *denA* and *denB* genes, respectively, and prevent the degradation of T4DNA with cytosine. In T4EDD, about 95% of the hydroxymethylcytosine will be replaced with cytosine. To isolate *Paf* mutants, we used the double mutant am E51, rDD2 (SNYDER, GOLD and KUTTER 1976) referred to as T4ED which is essentially T4EDD without a *denA* mutation. This phage will have less cytosine (SNYDER, GOLD and KUTTER 1976; WILSON, TANYASHIN, and MURRAY 1977; CARLSON and OVERVATN 1986) but is nevertheless totally sensitive to *Alc* function. The isolation of *Alc* mutants has been described (SNYDER, GOLD and KUTTER 1976). Nitrosoguanidine and 2-aminopurine mutageneses were by the procedures of

ADELBERG, MANDELL and CHEN (1965) and MILLER (1972), respectively.

Isolation of *Paf* mutants: The mutants we sought would propagate T4ED with cytosine DNA even if the phage were genotypically *alc*⁺. Since the phage would kill the host, regardless, we used a "nibbled colony" procedure. In a preliminary test with the permissive host, K803, we found that 10⁶ phage spread on a plate nibbled most colonies, did not reduce colony number, and would allow screening of about 500 mutagenized bacteria per plate. We used NTG mutagenized HR171 and B834 for the selections since they are both suppressorless and restrictionless. Any colony which gave a nibbled appearance was picked and retested by cross streaking with T4ED at 10⁷ per ml. Any mutants which passed this second test were colony purified at least twice and tested directly for their ability to plate T4ED as well as other mutants of T4.

Mapping *paf* mutations: To map the prototype *paf* mutation, *paf*-32, we made the original HR171 derived *Paf* mutant, HRP-1, auxotrophic for various markers by repeated cycles of NTG mutagenesis and ampicillin enrichment. Among the auxotrophs were an *Arg*⁻ mutant with an *arg* mutation in the *argECBH* cluster close to *rpoB* as determined by cotransduction with rifampin resistance. Sometimes, streptomycin-resistant derivatives were selected on streptomycin plates. These strains were crossed with various Hfr strains with different origins and directions of transfer at a ratio of 2 F⁻ to 1 Hfr and allowed to sit at 37°C without shaking for 1.5 hr. The recombinants prototrophic for various markers were selected, where necessary counterselecting the donor with streptomycin. The recombinants were purified and tested for the *paf* mutation by cross-streaking with T4ED. P1 transduction was as described (MILLER 1972), except that the bacteria were washed twice with saline containing 10⁻³ M sodium citrate, before plating. The *E. coli* K-12 map positions were taken from BACHMANN (1983).

Agarose gel electrophoresis and restriction nuclease digestions of T4 DNA: To prepare T4 DNA to test the cytosine content, we infected mid-log bacteria in M9S media as described (KUTTER and SNYDER 1983). After 1.5 hr the cells were lysed with chloroform and the phage concentrated by centrifugation and purified on a CsCl step gradient and dialyzed against M9. The T4 DNA was extracted with phenol in 0.01 M Tris-Cl pH 8, 0.001 M EDTA, and dialyzed against H₂O. The DNA was digested with restriction nucleases (IBI) using the supplied buffers and the digested DNAs were electrophoresed overnight in 0.1 M Tris-borate, 2 mM EDTA (pH 8.3), at 25 mV in submerged 0.7% agarose gels. The gels were stained with ethidium bromide and photographed on a UV transilluminator.

Protein labeling and polyacrylamide gel electrophoresis: *E. coli* in M9 media with 100 µg/ml of all 20 amino acids except methionine were infected at an m.o.i. of 10 at 30°. The proteins were labeled by adding 1 ml of infected cells to 10 µCi of carrier-free [³⁵S] methionine at 30 min after infection and the labeling stopped by adding ice to tubes at 33 min. The cells were lysed and electrophoresed as described (LAEMMLI 1970). The gels were fixed with 12% trichloroacetic acid, stained with Coomassie blue, dried, and autoradiographed.

Unfoldase assays: *E. coli* in M9S media were infected at 30° at an m.o.i. of 5. At the times indicated, 5 ml were added to tubes on ice containing 0.5 ml of 1 mg/ml chloramphenicol dissolved in H₂O, centrifuged, and lysed as described (SIROTKIN, WEI and SNYDER 1977) in a procedure based on an earlier published report (TUTAS, WEHNER and KOERNER 1974).

Viscosity of the lysates was estimated from the time, in seconds, for a 1-ml solution to flow through a 20 gauge needle 4.5 cm long. It is expressed as the ratio of this rate to the rate of flow of the uninfected cell lysate which was about the same as H₂O.

RESULTS

Isolation of Paf mutants of *E. coli*: To better understand how the *unf/alc* gene product of T4 blocks transcription of cytosine DNA, we attempted to isolate permissive *E. coli* mutants which could propagate cytosine-T4 even though the phage were genotypically *alc*⁺. Because T4 with cytosine DNA would kill such mutants, it was necessary to screen for "nibbled colonies" on a plate spread with T4ED as described in MATERIALS AND METHODS. We found permissive mutants at a fairly high frequency (about 1 per 10,000) and isolated nine such mutants, six from an *E. coli* K-12 strain, and three from the *E. coli* B strain. An amber suppressor mutation would cause the T4 to replicate with hydroxymethylcytosine and would therefore make the host permissive. Three of the mutants were found to have amber suppressors and were discarded. The other six *E. coli* mutants did not propagate phage with amber mutations. In particular, they would not propagate T4 with only the amber mutation in gene 56 without the accompanying *denB* deletion of T4ED. This suggests that, in these mutants, the phage are replicating with cytosine DNA since their DNA is sensitive to endo IV (KUTTER *et al.* 1975), the cytosine specific endonuclease which is the product of the *denB* gene.

To prove that the phage are multiplying with cytosine in the mutant hosts, we tested the cytosine content of the DNA of the phage, directly. We used the fact that glucosylated hydroxymethylcytosine containing T4 DNA is insensitive to most restriction nucleases. We purified the DNA from T4EDD phage grown for one generation on one of the mutants, *E. coli* BMP1 (see Table 1), and digested it with two different restriction nucleases (Figure 1, lanes C and H). The DNA was as completely digested as DNA from T4EDD with an *alc* mutation propagated on the parent *E. coli* which is shown for comparison (Figure 1, lanes D and I). We also show for comparison the digestion of the DNA of T4ED also propagated in BMP1, which is noticeably less sensitive to the restriction nucleases (Figure 1, lanes B and G), because it has substantially less cytosine. The T4ED phage are nevertheless sensitive to Alc function and will only multiply on a wild-type host if they are genotypically *unf/alc*⁻. We conclude that the host mutations have not affected the cytosine content of the phage DNA but, instead, are preventing the *unf/alc* gene product from blocking the transcription of the DNA even though the DNA has cytosine. We have not excluded the possibility that a DNA modi-

TABLE 1
Bacterial strains

Strain	Relevant characteristics	Source
<i>Hfr</i> Strains		
KL16-99	transfers counterclockwise 61' <i>recA1</i>	CGSC ^a
JEF-8	transfers counterclockwise 7' <i>metB1 carB8</i>	CGSC
KL227	transfers counterclockwise 7' <i>metB1</i>	CGSC
JDH-12	<i>hsdR2</i> derivative of JEF-8, <i>paf-32 met</i> ⁺ , <i>carB8</i>	This work
KDH-3	<i>hsdR2</i> derivative of KL227 <i>metB1</i>	This work
KDH-5	KDH-3 with <i>metA28</i> mutation from AB1932(CGSC) and a spontaneous <i>rif</i> mutation	This work
KDH-6	KDH-3 with <i>argECBH</i> , <i>paf-32</i> , and a spontaneous <i>rif</i> mutation	This work
<i>F'</i> Strains		
KL727	F'110/JC1553	CGSC
<i>F</i> ⁻ Strains		
AB468	$\Delta(gpt-proA)62$, <i>hisG4</i> , <i>purD13</i> , <i>supE44</i>	CGSC
ADS-1	an <i>hsdR2</i> , <i>sup</i> ^o derivative of AB468, <i>purD13</i> , <i>his</i> ⁺ , $\Delta(gpt-proA)$	This work
ADS-2	a <i>recA1</i> derivative of ADS-1 <i>pro</i> ⁺	This work
ADS-3	a derivative of ADS-1 with <i>recA1</i> , <i>pas-32</i> and <i>argECBH</i>	This work
HR171	<i>hsdR</i> ⁻ , <i>hsdM</i> ⁺ , <i>sup</i> ^o , K-12 strain	HELEN REVEL
B834	<i>hsdR</i> ⁻ , <i>hsdM</i> ⁻ , <i>sup</i> ^o , B strain, <i>met</i> ⁻	WOOD (1966)
K803	<i>hsdR</i> ⁻ , <i>hsdM</i> ⁻ , <i>supE</i>	WOOD (1966)
LCK8	<i>hsdR2</i> , <i>zii-202</i> Tn10	CGSC
BMP1	B834 with <i>paf-32</i> mutation from Hfr JDH-12 $\times$ B834 cross, <i>met</i> ⁺	This work
HRP1	HR171 with <i>paf-32</i> mutation	This work

^a CGSC: *coli* Genetic Stock Collection, B. BACHMANN, Yale University.

fication is induced in the mutants which, either because of its nature or because it is very localized, does not inhibit the restriction endonucleases even though it makes the DNA insensitive to Unf/Alc function. However, we consider this very unlikely. We shall subsequently refer to the mutations which make the cells permissive for *alc*⁺ cytosine T4 as *paf* mutations for prevent Alc function, although we are not certain that they directly affect Alc function. The mutant bacteria will be referred to as Paf mutants and the permissive and restrictive phenotypes will be referred to as the Paf and Paf⁺ phenotypes, respectively.

FIGURE 1.—Digestion of DNA from T4EDD propagated on *E. coli* with the *paf*-32 mutation by two different restriction endonucleases. Lanes A and F, T4EDD propagated in *E. coli* K803; lanes B and G, T4ED propagated in *E. coli* BMP1 (Paf); lanes C and H, T4EDD in BMP1; lanes D and I, T4EDD *alc*10 in *E. coli* HR171; lanes E and J, T4EDD *alc*10 in HRP1 (Paf). Lanes A→E: *Eco*RI; lanes F→J: *Hind*III.

The *E. coli paf* mutations seem to differ greatly in their efficacy. Some Paf mutants gave barely visible plaques and a reduced plating efficiency with cytosine T4, while two mutations, *paf*-32, isolated in the K-12 strain, and *paf*-75, isolated in the B strain, cause T4ED to form fairly large plaques and give almost 100% plating efficiency. This reflects the high rates of late protein synthesis from *alc*⁺ cytosine T4 DNA in at least one of the latter two mutants (see Figure 2 below). However, even the *paf*-32 and *paf*-75 mutations do not make *E. coli* as permissive for T4EDD as an *alc* mutation in the phage as evidenced by a smaller plaque size. Apparently, the *paf* mutations are not able to fully overcome the effects of higher substitution with cytosine. So far, we have detected no growth differences between Paf mutants and their parents.

TABLE 2
Transductional mapping of *paf*-32^a

Donor ^b	Recipient	Phenotype	Number
Cross I			
JDH-12 <i>paf</i> -32	KDH-5	Met ⁺ Rif ^R Paf ⁺	64
	<i>metA rif</i>	Met ⁺ Rif ^R Paf	0
		Met ⁺ Rif ^S Paf ⁺	0
		Met ⁺ Rif ^S Paf	51
Cross II			
KDH-3	KDH-6	Arg ⁺ Rif ^R Paf	134
	<i>paf</i> -32, <i>arg</i> ECBH	Arg ⁺ Rif ^R Paf ⁺	1
	<i>rif</i>	Arg ⁺ Rif ^S Paf	0
		Arg ⁺ Rif ^S Paf ⁺	111

^a Three-factor transductional crosses to determine the map position of the *paf*-32 mutation. The strains used are described in Table 1 and constructed as in MATERIALS AND METHODS.

^b Cross I, selection for Met⁺; cross II, selection for Arg⁺.

Mapping *paf* mutations: We performed Hfr crosses to determine the approximate map position of one *paf* mutation, *paf*-32. The *E. coli* K-12 strain, HR171, in which *paf*-32 was isolated, was a prototroph so we first made it a multiply marked strain as described in MATERIALS and METHODS. This multiply marked strain was crossed with various Hfr strains with different directions and origins of transfer. The various prototrophic recombinants were selected and tested for the *paf* mutation and the other markers. In a number of Hfr crosses, the *paf* mutation showed close linkage to an *arg* mutation at 90 min and we refined the mapping of the *paf*-32 mutation with P1 transductions selecting for various markers in this region. The *paf*-32 mutation showed about 12% cotransduction with *metB* and about 48% cotransduction with *purD*. Since *purD* and *metB* are only about 1% cotransducible with each other (data not shown), we concluded that the *paf*-32 mutation is between *purD* and *metB*.

Two of the genes for RNA polymerase, *rpoB* and *rpoC*, lie between *purD* and *metB* at 90 min. One of these, *rpoB*, determines rifampin resistance. To determine the map position of the *paf* mutation with respect to rifampin resistance, we performed the transductional crosses shown in Table 2. In the first cross, the donor had the *paf*-32 mutation and the recipient had a spontaneous rifampin resistance mutation and a *metA* mutation, clockwise of *rpoB* at about 91 min. All 51 of the 115 Met⁺ transductants we tested which were rifampin sensitive were also Paf. Furthermore, all 64 transductants which were still rifampin resistant were Paf⁺. Therefore, none of the transductants tested were recombinant for the rifampin resistance and *paf* markers, so the two markers must be very closely linked.

The close linkage between *paf*-32 and rifampin resistance was also apparent when we selected mark-

ers on the other side of rifampin resistance. In the second cross shown in Table 2 the donor was a prototroph and the recipient had the *paf*-32 mutation, a spontaneous rifampin resistance mutation, and a mutation in the *arg* ECBH cluster. Only 1 of the 246 Arg⁺ transductants was recombinant for the rifampin resistance and *paf* mutations, further evidence that they are very closely linked. We conclude that the *paf*-32 mutation is very close to, and probably in, the *rpoB* gene of *E. coli*.

Another indication that *paf* mutations are in *rpoB* came from testing the Paf phenotype of some spontaneous and induced rifampin-resistant mutants. We isolated 50 each of 2-aminopurine induced, nitrosoguanidine-induced, and spontaneous rifampin-resistant mutants of *E. coli* HR171. Of these, about 10% of the 2-aminopurine-, and 10% of the nitrosoguanidine-induced mutants showed a weakly Paf phenotype. While we have not excluded the possibility of second site mutations, we think this high frequency argues that a subset of 2-aminopurine- and nitrosoguanidine-induced rifampin resistance mutations inadvertently also cause a weakly Paf phenotype. However, none of the six mutants isolated for the Paf phenotype was rifampin resistant and the phenotype of the rifampin resistant mutants is very weak compared to the some of Paf mutants we isolated directly.

We also mapped the *paf*-75 mutation to determine if other *paf* mutations lie in the 90-min region. Conveniently, *E. coli* B834, the parent of the Paf-75 mutant, has a *met* mutation in the *rpoB* region probably *metA* based on its proximity to other markers (data not shown). When the Paf-75 mutant was crossed with an Hfr strain which was Paf⁺, about 70% of the Met⁺ recombinants were Paf⁺, indicating that the *paf*-75 mutation is also in the 90-min region of the *E. coli* genetic map. To determine if two of the independently isolated *paf* mutations are closely linked, we crossed Hfr JDH-12 (*paf*-32) with the Paf-75 mutant and tested 96 Met⁺ recombinants for the Paf phenotype. All were Paf mutants indicating that *paf*-75 and *paf*-32 are very closely linked.

***E. coli paf* mutations are dominant in merodiploids:** To determine whether *paf* mutations are dominant or recessive over the wild-type allele, we used the F' factor F110 which covers the *rpoB* region (Low 1972). First, we crossed the F' factor into ADS-3, a RecA⁻ derivative of ADS-1 into which previously we had transduced *paf*-32 and an *arg*ECBH mutation. All of the Arg⁺ recombinants were Paf suggesting that the *paf*-32 mutation is dominant over the wild-type allele. However, there is no assurance that the wild-type *paf* allele is on the F' factor, although we could induce spontaneous rifampin resistance mutations in F'110 containing merodiploids which could

FIGURE 2.—Synthesis of T4 late proteins after infection of *E. coli* BMP1 (Paf). Lane A, T4 wild type on a *met*⁺ derivative of B834; lane B, T4EDD on B834 *met*⁺; lane C, T4EDD *alc*2 on B834 *met*⁺; lane D, T4EDD on BMP1. In lane B all of the bands are due to early proteins. The proteins were labeled after infection and electrophoresed as in MATERIALS AND METHODS.

be transferred to *recA*⁻, F⁻, recipients by conjugation. To be certain that both the *paf*-32 allele and the wild-type allele were present in the merodiploids, we crossed the *paf*-32 mutation onto the F' factor and then mated it into a strain with the wild-type allele in the chromosome as described in MATERIALS AND METHODS. All eight of the merodiploids we tested which had the *paf*-32 mutation on the episome and the wild-type allele in the chromosome showed the Paf phenotype confirming that the Paf phenotype is dominant.

***E. coli paf* mutations prevent the Alc-induced block in T4 late gene expression from cytosine DNA:** Because *alc*⁺ T4 with cytosine DNA can form plaques on an *E. coli* Paf mutant, the *paf* mutation must be overcoming the block in T4 late gene expression due to the *unflalc* gene product. To determine how completely the block is relieved, we performed experiments such as the one shown in Figure 2. We infected the Paf mutant, *E. coli* BMP1, and pulse labeled proteins with [³⁵S]methionine late in infection at the time indicated. The effect of the host *paf* mutation was very dramatic. The rate of late protein synthesis was essentially the same whether the phage were *unfl*

alc⁻ or *unf/alc*⁺ (compare lane D to lane C). Therefore, the *E. coli paf* mutation seems to overcome completely the effect of the *unf/alc* gene product on the late transcription of T4 DNA with cytosine. For purposes of comparison, we also infected with wild-type phage and phage which made cytosine DNA but were *Alc*⁻ and we infected both the *Paf* mutant, BMP1, and its *Paf*⁺ parent, B834. As reported (SNYDER, GOLD and KUTTER 1976), the T4EDD phage made essentially no late proteins in the *Paf*⁺ strain (lane B), and the *alc2* mutation permitted normal rates of late gene expression (compare lane C to lane A). The *paf* mutation has little or no effect on the timing of T4 early gene expression from glucosylated hydroxymethylcytosine DNA (data not shown), which is important for the interpretation of experiments discussed immediately below. It also has no effect on late gene expression by cytosine T4 with the *alc2* mutation or wild-type T4 (data not shown).

We were somewhat surprised by the completeness with which *paf* mutations overcame the block in transcription from cytosine DNA, since the T4EDD phage make significantly smaller plaques if they are *unf/alc*⁺ than *unf/alc*⁻. However, a further experiment revealed that the T4EDD phage which were *unf/alc*⁺ exhibited a severe defect in early gene expression from cytosine DNA, even in a *Paf* mutant host. In the experiment shown in Figure 2, the infecting phage had glucosylated hydroxymethylcytosine DNA, and only the progeny DNA had cytosine. If we prepared the parent phage on a *Paf* mutant so they had cytosine DNA, and used them to infect a *Paf* mutant the *unf/alc*⁺ phage showed a severe defect in early gene expression relative to the phage which were *unf/alc*⁻ (data not shown). Apparently, the *paf* mutation is better able to overcome the effect of the *unf/alc* gene product on late than early transcription. This result also confirmed the conclusion of KUTTER and her collaborators (1981) that the *unf/alc* gene product can block T4 early transcription from cytosine DNA.

***E. coli Paf* mutants are delayed in the unfolding of their nucleoid after infection:** If the *Unf* and *Alc* phenotypes are but different manifestations of the same activity, then *paf* mutations might also prevent the *Unf* phenotype of the *unf/alc* gene product. In the experiment shown in Figure 3, we infected the *Paf* mutant, BMP1, and its parent, B834, with T4EDD which are genotypically *unf/alc*⁺. At the times shown, the cells were chilled, concentrated, and lysed as in MATERIALS AND METHODS. The viscosity of each lysate was measured to determine the time of unfolding of the bacterial nucleoid. It is apparent that the *paf* mutation significantly delayed the unfolding reaction, although the effect was not as absolute as when the cells were infected with the *Alc* mutant, *Alc 2*, which

FIGURE 3.—Unfolding of the bacterial nucleoid after infection of *E. coli* BMP1 (*Paf*). The relative viscosity of lysates was determined as in MATERIALS AND METHODS. T4EDD on B834: (○—○); T4EDD on BMP1: (△—△); T4EDD *alc2* on B834: (□—□).

was included in the same experiment. We have also determined the effect of the *paf*-75 mutation on the unfolding reaction. Again, the unfolding was significantly delayed relative to the *Paf*⁺ control (data not shown). Therefore, two independent *paf* mutations both caused a delay in unfolding of the nucleoid, and we conclude that the *Unf* phenotype of the *unf/alc* gene product also is affected by host *paf* mutations.

DISCUSSION

We have isolated *E. coli* mutants which can propagate T4 with cytosine DNA even if the phage are genotypically *alc*⁺. These mutations almost totally prevent the *unf/alc* gene product from blocking the transcription of the T4 late genes from cytosine DNA and we have named them *paf* mutations for prevent *Alc* function although we do not know that they directly prevent its action. We have mapped two *paf* mutations to the 90-min region very close to rifampin resistance mutations in the *rpoB* gene. Because of their map position and the observation that some rifampin resistance mutations seem to confer a weakly *Paf* phenotype, we think it most likely that *paf* mutations are in the *rpoB* gene encoding the β subunit

of RNA polymerase. We have isolated *paf* mutations in both an *E. coli* K-12 strain, HR171, and a B strain, B834, suggesting that *paf* mutations can be isolated in most strains of *E. coli*. Unfortunately, however, both of these strains could have the K-12 *rpoB* gene region since B834 is a *lamB* transductant from K-12 (ARBER and LATASTE-DORELLE 1961; WOOD 1966) and the *rpoB* gene may have cotransduced since it is closely linked. From the way they were isolated, it is difficult to estimate the frequency of *paf* mutations. However, since some of the permissive mutants had amber suppressor mutations instead of *paf* mutations we think they are about as frequent as amber suppressors which would mean that several base pair changes can cause the Paf phenotype.

Assuming that *paf* mutations are in RNA polymerase, how could RNA polymerase mutations prevent the *unfIalc* gene product from blocking transcription from cytosine DNA? One obvious explanation is that the *unfIalc* protein must bind to RNA polymerase to exert its action, and that *paf* mutations alter RNA polymerase so as to preclude this binding. We do not think it paradoxical, in terms of this hypothesis, that the *unfIalc* protein has been shown to bind to DNA, since it might bind to both DNA and RNA polymerase. Alternatively, the *paf* mutations may act indirectly, for example by allowing the RNA polymerase to transcribe cytosine DNA in spite of the *unfIalc* gene product. While it is conceivable that *paf* mutations prevent the synthesis of the *unfIalc* protein, we think this highly unlikely since the synthesis of other T4 early proteins is not affected. Moreover, even if the synthesis of the *unfIalc* gene product were delayed, we would nevertheless expect it to be present late in infection. We reason that even though the Unfolding phenotype is delayed, it does eventually occur. If this delay reflects a delay in the synthesis of the *unfIalc* gene product, it would be present at late times after infection when the Alc phenotype occurs. Thus we strongly prefer the interpretation that *paf* mutations prevent Alc and Unf function by preventing the activity of the protein rather than by delaying its synthesis.

Unfortunately, speculation about how *paf* mutations might prevent the action of the *unfIalc* gene product is hampered by our lack of understanding of how the *unfIalc* protein acts to block transcription. There are peculiar features of the block in transcription which suggest that the *unfIalc* protein does not merely change promoter specificity so that RNA polymerase can no longer initiate from promoters with cytosine. For one, the *unfIalc* protein can block initiation from a variety of genes serviced by different types of promoters provided the DNA has cytosine (KUTTER *et al.* 1981; PEARSON and SNYDER 1980). Furthermore, the late genes of T4, which are sensitive

to the *unfIalc* gene product are serviced by promoters which have no cytosine in the consensus sequence (CHRISTENSEN and YOUNG 1982). Finally, there is evidence that the *unfIalc* protein can block the propagation as well as, or instead of, the initiation of transcription (PEARSON and SNYDER 1980).

The existence of *paf* mutations also does little to help explain the relationship between the Alc and Unf phenotypes except to further confirm that they are related. While the effect of host *paf* mutations on T4 late gene expression is very dramatic, the effect on the host nucleoid unfolding reaction, while seemingly real, is less dramatic. The host nucleoid unfolds, but with only about a 2–3-min delay relative to the wild type in the two mutants we tested. We were not particularly surprised that *paf* mutations have a more dramatic effect on the Alc than on the Unf phenotype of the *unfIalc* gene product. The same often is true of *unfIalc* mutations induced in the bacteriophage. Only the most completely inactivating *alc* mutations show the Unf[−] phenotype. Apparently, the Alc phenotype is more sensitive to the level of Unf/Alc activity than the Unf phenotype.

Any proposed mechanism of action for the *unfIalc* gene product must explain how the same gene product can cause these two phenotypes: the Alc phenotype of blocking transcription from cytosine DNA; and the Unf phenotype of unfolding the host nucleoid. One phenotype may be a direct consequence of the other. Blocking transcription may cause the unfolding of nucleoids. There is a precedent for such an effect since rifampin also causes the unfolding of the bacterial nucleoid (PETTIJOHN and HECHT 1973) or at least destabilizes it *in vitro* (SINDEN and PETTIJOHN 1981). However, *unfIalc* mutations may cause at most a short delay in the shutoff of most *E. coli* transcription (TIGGES, BURSCH and SNUSTAD 1977; KOERNER and SNUSTAD 1979) although this is not clearly established. Alternatively, unfolding nucleoid-like structures may block certain types of transcription. If the latter prevails, our evidence that RNA polymerase mutations can affect the unfolding reaction suggests that RNA polymerase may play a direct role in maintaining nucleoid structure.

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