DNA Polymerase I-Dependent Mutants of Coliphage Lambda

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Mutagenized lysates of bacteriophage lambda were screened for mutants unable to plate on DNA polymerase I-deficient (*polA*⁻) hosts. The mutants obtained were all recombination deficient (*red*⁻). These mutants, like *red*⁻ and *gam*⁻ mutants previously isolated by others, grow more poorly than wild-type λ even on *polA*⁺ hosts (burst size 14 to 30% of wild-type λ.) In a *polA*⁻ host, the burst size of *red*⁻ and *gam*⁻ mutants is reduced an additional five- to tenfold, and lysis is delayed. Wild-type λ grows normally in *polA*⁻ hosts. Neither λ*N*⁻ⁿⁱⁿ (which doesn't express *red* or *gam*) nor λ*bio* phages (from which all or part of the *red-gam* region is deleted) form plaques on *polA*⁻ hosts. Apparent revertants, able to plate on *polA*⁻ hosts, have been selected from both λ*N*⁻ⁿⁱⁿ and λ*bio*. Those derived from *N*⁻ⁿⁱⁿ seem to be *N*⁻ⁿⁱⁿ *cro*⁻ mutants; whereas those coming from λ*bio* have a new bypass mutation (*pas*) that lies between genes *P* and *Q*.

DNA polymerase I is one of three DNA polymerases in *Escherichia coli* K-12. DeLucia and Cairns (7) isolated an *E. coli* mutant (P3478) which has greatly reduced DNA polymerase activity yet grows normally (7, 11). The mutation (*polA1*) causing polymerase deficiency in strain P3478 allows growth of many phages and maintenance of some plasmids, but not of the *colE1* factor (15). Bacteriophage λ can grow on *polA*⁻ strains of *E. coli*. But mutants deficient in functions (*red* and *gam*) needed for recombination or for growth on *recA*⁻ hosts fail to plate on *polA1* strains (23). More specifically, a phage must be either *red*⁺ or *red*^β⁺ to recombine in a *recA*⁻ host; to grow on hosts that are *recA*⁻ *recB*⁺ *recC*⁺, it must be either *red*^α⁺ *red*^β⁺ or *gam*⁺; whereas growth on *polA1* hosts requires that it be *red*^α⁺ *red*^β⁺ *gam*⁺. We report here isolation of additional *red*⁻ mutants (picked as *polA* dependent), characterization of their growth on polymerase-deficient hosts, and genetic studies of further mutations that allow such mutants to bypass the *polA* requirement.

MATERIALS AND METHODS

Bacterial strains. Table 1 lists various derivatives of *E. coli* K-12 used in these studies. The extent of deletion or substitution of λ genes in several defective lysogens and λ*bio* phages is shown in Fig. 1 and 2. Previously isolated *red*⁻ and *gam*⁻ mutants were from the collection of J. Zissler.

Media and crosses. Media have been described previously (20). Phage crosses were carried out by lytic growth on the appropriate host after adsorption

in 10 mM MgSO₄ for 20 min at room temperature. Cells were infected at a multiplicity of 5 for each parental phage. The infected cells were diluted 50-fold into tryptone broth, incubated 90 min at 37°C, treated with CHCl₃, and plated.

Deletion mapping. Bacteria were grown to 2 × 10⁸/ml and resuspended in 10 mM MgSO₄. λ*bio*22-5 *cI*857 *pas*2 (for crosses where *h* recombinants were selected) or its *Pam*500 derivative (for crosses where *P*⁺ recombinants were selected) was adsorbed at a

TABLE 1. Bacterial strains

Strain	Characteristics	Source or reference
W3110	<i>thy</i> ⁻	(7)
P3478	<i>thy</i> ⁻ <i>polA1</i>	Mutant of W3110 (7)
PR3478	<i>thy</i> ⁻	Revertant of P3478 (4)
KL1699	Hfr KL16 <i>recA1</i>	B. Low
BC141	<i>thy</i> ⁻ <i>polA1</i> <i>trpAam</i> 9761 <i>supD</i>	B. Coukell
BC143	<i>thy</i> ⁻ <i>polA1</i> <i>trpAam</i> 9761 <i>supE</i>	B. Coukell
BC145	<i>thy</i> ⁻ <i>polA1</i> <i>trpAam</i> 9761 <i>supF</i>	B. Coukell
HS325	<i>polA4</i> Sm ^r	Sm ^r mutant of P62 (13)
HS326	<i>polA4</i> <i>supF</i> Sm ^r	Cross HS325 × Hfr <i>supF</i>
LG102	P2 lysogen of HfrH	(2)
C600	<i>thr</i> ⁻ <i>leu</i> ⁻ <i>thi</i> ⁻ <i>supE</i>	(5)
C600-15	<i>thr</i> ⁻ <i>leu</i> ⁻ <i>thi</i> ⁻ <i>supE</i> <i>groPA15</i>	C. Georgopolous (11)
W3350	<i>galK2</i> <i>galT1</i>	(3)
R594	<i>galK2</i> <i>galT1</i> Sm ^r	(3)
HS435	<i>galK2</i> <i>galT1</i> (λ <i>cI</i> 857 <i>ind</i> ⁻)	Lysogenization of W3350
CR63	Resistant to λ, sensitive to λ <i>h</i>	(18)

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FIG. 1. Substitution map of λ bio (by the method of Signer et al. [21] and Zissler et al. [23]). The substitutions of λ bio11 and λ bio1 (not shown) terminate within the *gam* gene, like that of λ bio22-5.

FIG. 2. Deletions used in mapping *pas2*. Data on rescue are from Table 5. In strain S1014, the deleted DNA is substituted by bacterial DNA (*galM3* substitution). Relative positions of R906, SA307, and S1014 end points are from Pero (17). The fusion of *gal* DNA to *Q* gene DNA in S1014 and R901 is derived from the 5061 deletion of Shapiro and Adhya (19). MS501 is described by Shapiro and Adhya (19). Strain SA538 (used in Table 5) is a derivative of R906 carrying the *h* mutation. Strain S947 (used in preparing the λ bio22-5 *cI857 pas2* Ram60 parent for Table 5) is R901 bearing the Ram60 and Aam121 mutations.

multiplicity of 1 at room temperature for 20 min. The infected cells were diluted 10-fold into 10 mM MgSO_4 , irradiated with UV, diluted 10-fold into tryptone broth, and grown for 90 min at 37 C, when CHCl_3 was added. For crosses where R^+ recombinants were selected, λ bio22-5 *cI857 pas2* Ram60 was grown in plate lysates on the various hosts.

R^+ and P^+ recombinants were selected on *Su*⁻ bacteria (either W3350 or the P2 lysogen LG102), and *h* recombinants were selected on CR63. Recombinants that were *am*⁺ or *h* and *Spi*⁻ (thus, still carrying the *bio22-5* substitution) were tested for loss of *pas* by plating on P3478.

The λ bio22-5 *cI857 pas2* Pam500 phage was isolated by plating λ bio22-5 *cI857 pas2* on the *groP supE* strain C600-15, on which λP^+ fails to plate (11). The λ bio22-5 *cI857 pas2* Ram60 was obtained by induction

of a lysogen made by infecting S947 (Fig. 2, legend) with λ bio22-5 *cI857 pas2*.

Isolation of λ mutants. HS435 was grown in tryptone broth at 30 C to 2×10^8 cells per ml and transferred to 42 C for 15 min to derepress the prophage. Cells were pelleted by centrifugation and resuspended in 50 mM Tris-maleate buffer (pH 6.1) containing nitrosoguanidine (500 $\mu\text{g}/\text{ml}$) for mutagenesis. After incubation at room temperature for 30 min, the cells were centrifuged, washed, resuspended in the same volume of 10 mM MgSO_4 , diluted 100-fold into tryptone broth, divided into portions that were placed in separate tubes, and shaken at 37 C for 2 h. Then CHCl_3 was added, and the mutagenized phages were plated on W3110 at 30 C. Individual plaques were then picked up with toothpicks and stabbed onto lawns of P3478 and W3110. One plaque that showed lysis on W3110 but little or none on P3478 was selected from each tube. Thus, all isolates are independent. The lysate from the mutagenized cells contained 2 to 3% clear mutants. Seventeen polymerase-dependent mutants were isolated from about 1,500 plaques tested.

Phage growth. To measure burst sizes (Table 2), 2×10^8 cells grown in tryptone broth were centrifuged, suspended in 10 mM MgSO_4 , and starved for 30 min at 37 C. Then cells were infected with phages at a multiplicity of 5. This mixture was diluted 100-fold into prewarmed tryptone broth and incubated for 90 min at 37 C. CHCl_3 was added, and lysates were plated on C600.

RESULTS

Some properties of the mutants. Generally, our mutants resemble previously described *red* mutants (23) in making smaller plaques and in

TABLE 2. Effect of *polA* mutation on burst size of λ mutants

Phage ^a	Host (genotype)	
	W3110 (<i>polA</i> ⁺)	P3478 (<i>polA1</i>)
λ +	192	150.0
λ red708 ^b	35	3.7
λ red719 ^b	41	3.6
λ red739 ^b	50	8.0
λ red740 ^b	25	3.6
λ red743 ^b	40	4.5
λ bio69	34	5.6
λ bio22-5	57	6.6
λ redA324 ^c	42	13.0
λ redB113 ^c	51	11.0
λ redC314 ^c	27	3.8
λ gam210	27	5.0

^a All phages bear the *cI857* mutation.

^b Mutants isolated in this work. Classified *red* on the basis of Table 3 and similar experiments.

^c Mutants described by Zissler et al. (21). Mutants *redA324* and *redC314* belong to separate complementation classes, but both lie in the exonuclease gene *redα*. Mutant *redB113* lies in *redβ*.

having a lower burst size than wild-type λ , even on the *polA*⁺ host W3110. Most of the mutants produce turbid plaques at 37 C, whereas the parental *cI857* plaques are extremely clear. On *polA*⁻ hosts, the mutants formed no plaques, or very tiny plaques, depending on plating conditions. In 4 of our 17 mutants, polymerase dependence was reversible by an amber suppressor. These mutants formed good plaques on HS326 (which is *polA*⁻ *supF*) as well as on W3110 (which is *polA*⁺ *Su*⁻), but not on HS325 (which is *polA*⁻ *Su*⁻). They thus have the phenotype expected to result from an amber mutation in a gene required for growth on *polA*⁻, but not *polA*⁺, hosts.

Dependence of phage growth on *polA* gene.

Failure of λ mutants to plate on P3478 might result from bacterial mutations in P3478 other than *polA1*. Two lines of evidence indicated that *polA1* itself prevents growth of our mutants. (i) All our mutants plate on PR3478, which is a *polA*⁺ revertant of P3478 (4). (ii) Our mutants also plated on strains BC141, BC143, and BC145, which carry suppressors that restore the *PolA*⁺ phenotype to *polA1* mutants (13). Except for the four mutants that plate on HS326 (previous paragraph), the phage mutations should not themselves be suppressible in BC145.

Growth of mutants on *polA*⁻ hosts. When P3478 lysogenic for a mutant of our collection was heated to induce derepression, lysis was not complete until 105 min, whereas a W3110 lysogen of the same mutant lysed at 75 min (Fig. 3). A P3478 lysogen of the parental phage λ cI857 lysed normally at 75 min. Delayed lysis is seen also in one-step growth curves (Fig. 4). Production of mutant phages in the *polA*⁻ host is delayed about 10 min, and less total phage is found. In all mutants tested, the phage yield on *polA*⁻ is about 10% of that on *polA*⁺, although the parental *cI857* grows quite well on the *polA*⁻ strain (Table 2).

Effects of known λ genes. The product of λ gene *N* activates rightward transcription of genes *O*, *P*, and *Q* (which are essential for phage growth) as well as leftward transcription of a DNA segment that extends from *cIII* through *int* (Fig. 1) and includes genes *red* and *gam* (7). Certain mutations allow plaque formation on wild-type hosts by *N*⁻ phages (1, 6, 14), presumably because they increase *N*-independent rightward transcription. One such mutation, *nin5* (6), was employed in our experiment. Since *N* protein is needed for full expression of genes to the left of *N*, the phage *Nam7 am53 cI857 nin5* forms plaques on a nonpermissive host but is deficient in integration, excision, and recom-

FIG. 3. Lysis of induced lysogens. Strains grown on tryptone broth supplemented with thymine (10 μ g/ml) were brought to 2×10^8 cells per ml at 30 C, (time 0) transferred to 42 C for 15 min, and then incubated at 37 C. Optical densities were followed in a Klett-Summerson colorimeter. Symbols: $\times$, W3110 (non-lysogen); $\circ$, W3110 (λ cI857); $\bullet$, W3110 (λ cI857 red743); Δ , P3478 (λ cI857); $\blacktriangle$, P3478 (λ cI857 red743). λ cI857 red743 is an amber mutant.

bination. Since growth on *polA*⁻ hosts depends on products of genes to the left of *N*, this phage should not plate on a *polA*⁻ strain. Phages λ Nam7 *am53 cI857 nin5* and λ Nam7 *imm434 nin5* both failed to plate on P3478, but made minute plaques on the parental strain W3110. λ N⁺*nin5* phage plates normally on P3478.

To further localize those determinants needed for growth on *polA*⁻ hosts, we used various λ bio phages. Figure 1 shows the extent of phage DNA that has been substituted by bacterial DNA in each *bio* phage used. Of the phages shown, only λ bio7-20 formed plaques on P3478; *bio1*, *bio69*, *bio11*, *bio22-5*, *bio10*, and *bio252* did not. Thus, no genes lying within the *bio7-20* substitution (such as *int* or *xis*) are needed for growth on *polA*; whereas λ bio missing some or all of the *red* genes fail to plate. (Some λ bio with even more extensive substitutions, such as λ bio30-7 *nin5*, do plate on P3478 in our hands. Thus, the above statement applies only to λ bio whose substitution does not extend as far as the *N* gene. We will not try to explain here the properties of λ bio deleted for *N*.) Of three *red*⁻ mutants and two *gam*⁻ mutants

FIG. 4. Kinetics of phage production after induction. Cells treated as in Fig. 3, except that after 15 min at 42°C induced cultures were diluted 100-fold into prewarmed T-broth and incubated at 37°C. Samples were plated directly on C600 at the indicated time. Symbols: O, W3110 (λ cI857); ●, W3110 (λ cI857 red 719); Δ, P3478 (λ cI857); ▲, P3478 (λ cI857 red 719).

obtained from J. Zissler, none plated on P3478; in agreement with previous results (23). Burst size measurements for four of these mutants appear in Table 2.

Recombination deficiency of mutants isolated as polymerase dependent. We wanted to know whether the mutants we had picked were *red*⁻, *gam*⁻, or something else. Known *red*⁻ mutants rescue genetic markers from a "cryptic" λ prophage quite poorly (22) and were originally isolated on this basis. All our mutants were partially or completely deficient in such rescue ability. More quantitative results were obtained by crossing individual mutants with λ bio22-5 cI857 Pam500 in a *recA*⁻ host (Table 3). In λ bio22-5, the DNA segment from the attachment site to the *gam* gene is replaced by bacterial DNA (Fig. 1). This phage has lost the phage-specified recombination function completely and has concomitantly acquired the ability to grow on *E. coli* strains lysogenic for phage P2. Wild-type λ are sensitive to P2 inhibition (Spi⁺ phenotype), due to expression of any of several genes, delta, *red*, and *gam* (15), all of which are absent from λ bio22-5.

Thus, in the crosses of Table 3, only the P⁺

bio22-5 recombinants plate on the Su⁻ P2 lysogen LG102. All mutations tested abolished all detectable recombination and are thus *red*⁻. The formal possibility that they might lie in some previously undiscovered *red* genes is strongly restricted by the fact that the mutants fail to complement λ bio22-5 in the experiment of Table 3. Thus, they probably lie within the DNA deleted from that phage.

Bypass mutations allowing growth of the mutants in a *polA*⁻ host. When large amounts of λ bio or λ Nam7 *am53 cI857 nin5* are plated on P3478, plaques are found at a frequency of 10⁻⁵ to 10⁻⁶. These mutations must bypass rather than reverse the genetic blocks of the parental strains because the λ bio have no *red* or *gam* genes and λ Nam7 *am53* has two mutations inactivating gene *N*. One mutant from each source was studied further.

The bypass mutant of λ Nam7 *am53 cI857 nin5* makes minute plaques on P3478 as well as on W3110. It failed to plate on the *supE* strain C600 at 30 and at 42°C, but grew at 37°C. Unlike the parental λ Nam7 *am53 cI857 nin5* phage, the mutant could plate on the *recA*⁻ Su⁻ host KL1699.

The multiple mutant λ Nam7 *am53 cI857 nin5 cro*⁻ is known to plate on *recA*⁻ Su⁻ hosts and to be sensitive to low and high temperatures (5, 10). Therefore, we tested a phage of that genotype (bearing the *cro1* mutation, 5) on P3478 and found minute plaques. Hence, this kind of bypass mutation is probably identical to *cro*⁻. On the other hand, the bypass mutant of λ bio22-5 cI857 plated on both *supE* and Su⁻

TABLE 3. Effect of mutation on recombination^a

<i>red</i> parent ^b	Spi ⁻ P ⁺ recombinants ^c	
	Expt I	Expt II
<i>red</i> ⁺ <i>gam</i> ⁺	0.37	0.71
<i>gam</i> 210	0.13	NT
<i>red</i> 719 ^e	<0.01	<0.01
Revertant of <i>red</i> 719 ^e	NT	0.78
<i>red</i> 737 ^d	<0.01	NT
<i>red</i> 739 ^d	<0.01	NT
<i>red</i> 743 ^e	NT	<0.01

^a Cross: *bio22-5 Pam500* × *red* +. Selected recombinant: *bio22-5* +.

^b All phages carry the *cI857* mutation. The other parent was always *λ*bio22-5 *cI857 Pam500*.

^c Crosses performed in the Su⁻ *recA*⁻ host KL1699 as described in Materials and Methods. Cross lysates were plated on C600 for total phage and on LG102 for Spi⁻ P⁺ recombinants. Values listed are 100 × [(plaques on LG102)/(plaques on C600)]. NT, not tested.

^d Mutants isolated in this work.

^e Revertant selected for ability to plate on P3478.

hosts at any temperature, but failed to plate on the *recA*⁻ host KL1699. We will call this kind of bypass mutation *pas* and the particular mutation studied in detail was *pas2*.

Mapping the *pas* mutation. In crosses between *pas2* and *pas*⁺ phages (Table 4), recombinants selected as *N*⁺*P*⁺ usually display the *pas* character of the *P*⁺ rather than the *N*⁺ parent. Thus, the mutation should lie either immediately to the left of the *P* gene or else somewhere to its right.

More definitive localization was accomplished by selective crosses between *bio22-5 cI857 pas2* derivatives and various deletion prophages (Tables 4 and 5). Since these prophages all came from *pas*⁺ lysogens, each deletion strain should include either the *pas*⁺ allele or no allele at all at the *pas* locus. In each case, recombinants between a *bio22-5 cI857 pas2* derivative and the prophage are selected as phages bearing some genetic marker that can come only from the prophage, e.g., lysogens were superinfected with *bio22-5 cI857 pas2 Ram60*, and the progeny phage were plated on the *Su*⁻ host W3350 to select *R*⁺ phages. Since none of the prophages contains a complete set of genes needed for plaque formation, such phages can arise only by recombination. The *R*⁺ recombinants are then individually scored for the *pas* character.

Our failure to find *pas*⁺ recombinants in strains R901, RR905, and S1014 indicates that the *pas* locus is absent from these strains, whereas the occurrence of *pas*⁺ recombinants using SA307, SA538, R515, and R906 demonstrates that the locus is present in these cases. The positive results preclude any location outside the *P-R* region; the negative results further localize the mutation to a site between the 307 and 5061 deletion end points. Thus, *pas2* lies

TABLE 5. Deletion mapping of *pas2*^a

Deletion parent	λ genes present	Selected marker X ⁺	Fraction <i>pas</i> ⁺ recombinants among X ⁺ phage ^b
R515	(<i>int-R</i>)	<i>R</i> ⁺	16/32 ^c
S1014	(<i>int-P</i>), (<i>Q</i> ^Δ - <i>J</i>)	<i>R</i> ⁺	0/39 ^d
MS501	(<i>P-J</i>)	<i>P</i> ⁺	9/19
SA538	(<i>Q-J</i>)	<i>h</i>	12/30
R906	(<i>Q-J</i>)	<i>R</i> ⁺	20/24
SA307	(<i>Q-J</i>)	<i>R</i> ⁺	26/41
R901	(<i>Q</i> ^Δ - <i>A</i>)	<i>R</i> ⁺	0/24
RR905	(<i>A-J</i>)	<i>h</i>	0/29

^a Cross: *bio22-5 pas2 X*⁻ × (*deletion*) *X*⁺. Selected recombinant: *bio22-5 X*⁺.

^b Crosses performed as described in Materials and Methods. Only *Spi*⁻ recombinants are listed.

^c Initial selection on W3350. Among the 32 recombinants listed, 31 were *immλ* and was one *imm434 pas*⁺. Among *Spi*⁺ recombinants, 13 were *imm434* and 3 were *immλ*.

^d Initial selection on W3350. All 39 recombinants were *Spi*⁻ *immλ*.

either between *P* and *Q* or in the left half of the *Q* gene.

Our data do not prove that the *pas2* mutant differs from wild type at a single locus. It could be that several alterations are needed to permit growth on *polA*⁻ hosts. If any of these alterations lie outside the *galM3* substitution present in S1014, phenotypically wild-type recombinants could arise in the cross with that phage. Thus, within the sensitivity imposed by the number of recombinants tested, all components of *pas2* lie within the *galM3* substitution and thus between *P* and the middle of *Q*.

DISCUSSION

More than 90% of the phage production in a *polA*⁻ host requires the action of three phage genes *redα*, *redβ*, and *gam* and, therefore, of their protein products, exonuclease, β protein, and γ protein (Table 2, column 2). The delay in expression of late functions suggested by the lysis curves of Fig. 3 must be a consequence, perhaps very indirect, of the absence of some of these products. A reasonable working hypothesis might be that the primary effect is on DNA replication, and that expression of late genes is limited by the number of DNA copies. In that event, the *pas2* mutation might either influence DNA synthesis, or else alleviate the consequences of limited synthesis by increasing transcription of gene *Q*, whose product activates late protein synthesis. That question remains to be elucidated. We can say at least that the *pas* mutation does not reverse all effects of *red-gam*

TABLE 4. Phage crosses to map *pas2*^a

Unselected characters of <i>Spi</i> ⁻ <i>am</i> ⁺ recombinants	No. of recombinants when <i>Nam</i> parent was: ^b	
	<i>Nam7 imm434</i>	<i>Nam7 am53 immλ</i>
<i>pas</i> ⁺ <i>immλ</i>	12	35
<i>pas</i> ⁺ <i>imm434</i>	3	0
<i>pas2 immλ</i>	1	5
<i>pas2 imm434</i>	2	0

^a Cross: *bio22-5 + Pam 500 pas2* × + *Nam* + +. Selected recombinant: *bio22-5 N*⁺ *P*⁺.

^b Crosses were performed on strain C600. Progeny phage were plated on LG102 to select *Spi*⁻ *N*⁺ *P*⁺ recombinants, which were then tested for immunity and *pas2*. Parents with *immλ* carried the *cI857* mutation.

deletion, because λ bio22-5 *cI*857 *pas*2 remains sensitive to P2 inhibition and unable to grow on *recA*⁻ hosts.

The other type of bypass mutation, isolated from *Nam cIts nin* phages, resembles *cro*⁻ mutations (9, 10) by the following criteria: (i) high frequency of lysogenization (data not shown); (ii) inability of λ cI857 *cro*⁻ to form plaques at 30 or 42 C; (iii) growth of λ *Nam cro*⁻ on *recA*⁻ *Su*⁻ hosts. The *cro* gene product represses leftward transcription (17). *N*⁻ *cro*⁻ phages express genes of the *int*-*cIII* operon at a rate which is low but sufficient to allow growth on *recA*⁻ strains (5).

The results of studies with *red* and *gam* mutants imply that host DNA polymerase can partially replace the exonuclease and β and γ proteins for some aspects of phage development. The two systems are not equivalent in all respects, e.g., the recombination defect of *red*⁻ mutants is observed in *polA*⁺ hosts and, thus, is not reversed by host polymerase. Rather, host polymerase must participate in an alternative pathway that allows some replication of these viral mutants.

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