

## Interaction of P2 Bacteriophage with the *dnaB* Gene of *Escherichia coli*

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Received for publication 3 March 1975

The *dnaB* gene product of *Escherichia coli* is required for multiplication of temperate phage P2. At 37 C in *dnaB-ts* mutants, P2 will not plaque and gives a very small burst of progeny. P2 mutants have been isolated which can grow well enough to plaque under these conditions. This type of phage mutant is *cis* dominant, and one such mutant (P2 *rlb*<sub>1</sub>) has been mapped near the left end of the early gene B and to the right of the *cox*<sub>4</sub> (excision) mutation. The *rlb*<sub>1</sub> mutation does not lie at the replication origin, but may affect transcription in the early region, which includes the replication origin. It may also represent a site on the P2 DNA which interacts with the *dnaB* gene product.

The DNA of temperate phage P2 replicates unidirectionally from a defined origin (24). Replication of the P2 genome requires the *cis*-acting product of phage gene A (17), as well as the product of phage gene B (21), and a nonessential host gene, termed *rep* (6, 9). We would like to define all the gene products needed for P2 replication and report here the requirement for the *Escherichia coli dnaB* gene product, as well as the properties of a P2 mutant with an altered requirement for this gene product.

### MATERIAL AND METHODS

**Bacterial strains.** *E. coli* C-la is a fast-growing prototrophic derivative of *E. coli* C (23). *E. coli* C-1055 (27) is polyauxotrophic, streptomycin resistant, and is used as the standard indicator for P2 phage. K12SH28 is a thymidine phosphorylaseless prototroph, and FA22 is a *dnaB-ts* mutant derived from it (12). Since FA22 did not grow well at 30 C we isolated FA22-1 as a large colony former at 30 C. It grows well at 30 C but not at 42 C. CRT266 (*thr leu met arg dnaB-ts*) is described by Kohiyama et al. (14). CRT266R is a spontaneous temperature-resistant revertant of CRT266 which plates wild-type P2 normally at 42 C. H502 is a *thyA, uvrA, endI* derivative of *E. coli* strain C, and LD312 is a *dnaB-ts* mutant derived from it (11). C-1757 is an amber-suppressing derivative (*supD*) of the polyauxotrophic strain C-436 (26).

**Phage strains.** The strains previously reported are described in Table 1. P2 *vir*<sub>1</sub> *rlb*<sub>1</sub> was isolated after plating 10<sup>9</sup> P2 *vir*<sub>1</sub> on CRT266 lawn at 37 C. At this temperature the bacteria form a lawn, but the plating efficiency of P2 is only about 10<sup>-8</sup> compared to the efficiency of plating at 30 C. The mutation *rlb*<sub>1</sub> causes

P2 to plate on CRT266 at the same efficiency whether the temperature is 30 or 37 C. The recombinant strain P2 *rlb*<sub>1</sub> was isolated from a cross between P2 *vir*<sub>1</sub> *rlb*<sub>1</sub> and P2 *amb*<sub>116</sub>. The phenotype of P2 *rlb*<sub>1</sub> is the same on LD312 and FA22-1 as on CRT266. P2 *vir*<sub>301</sub> *rlb*<sub>1</sub> is a spontaneous immunity-insensitive mutant isolated after plating P2 *rlb*<sub>1</sub> on a P2 lysogenic strain. The CsCl<sub>2</sub> buoyant density difference (0.001 g/cm<sup>3</sup>) between P2 *vir*<sub>301</sub> *rlb*<sub>1</sub> and P2 *vir*<sup>+</sup> *rlb*<sub>1</sub> suggests that the *vir*<sub>301</sub> mutation is a small deletion. Compared with the small deletion P2 *vir*<sub>79</sub>, which differs from P2 *vir*<sup>+</sup> by 0.0012 to 0.0015 g/cm<sup>3</sup> and corresponds to a deletion of 1.2% of the P2 genome (3), the *vir*<sub>301</sub> deletion should be 1.2% or less.

**Media.** LB and LB agar (1) were used with the NaCl concentration reduced to 0.1 M. TPG-CAA is described by Lindqvist (21).

**One-step growth experiments.** One-step growth experiments to determine average burst sizes were performed as described by Six and Klug (25). Incubation temperatures are given in the table legends. Multiplicities of infection were chosen so as to infect most cells and usually ranged between 6 and 14. Phage yields were calculated per infected cell. In most experiments the timing of the phage yield assays depended on turbidity measurements indicating completion of lysis.

**Phage crosses.** Phage crosses were performed in *E. coli* C-la or *E. coli* C-1757 at 30 or 37 C using the procedure described by Lindahl (15). Phage were treated with UV to a survival of 50%. This treatment markedly increases the recombination frequency for P2.

**P2 DNA synthesis.** Measurement of the incorporation of [<sup>3</sup>H]thymidine into acid-insoluble material was determined using the procedure of Lindqvist (21). Cells were grown at 30 C with aeration in TPG-CAA supplemented with 10 μg of cold thymine per ml to a titer of 7 × 10<sup>7</sup> cells/ml and concentrated to 2 ×

$10^6$ /ml. One portion was treated with mitomycin C (60  $\mu$ g/ml) for 10 min at 30 C without aeration. The cells were collected by centrifugation, washed once with 1% saline, resuspended to volume in TPG-CAA plus 2  $\mu$ g of cold thymine per ml, and infected, and [ $^3$ H]thymidine was added (see Fig. 1 legend for details). At appropriate times, 0.1-ml aliquots were withdrawn from the cultures into 1 ml of cold trichloroacetic acid (10%). After 30 min in the cold, the precipitates were collected by centrifugation, and the pellets were resuspended in 0.5 ml of 1 M NaOH. The samples were incubated overnight at 37 C, neutralized with an equal volume of 1 M HCl, and precipitated with an equal volume of 20% trichloroacetic acid. The precipitates were collected by filtration onto glass filter pads (Whatman GF-A). Scintillation fluid was added, and counting was performed in a Packard Liquid Scintillation Spectrometer.

## RESULTS

**Growth of P2 on *dnaB* mutants of *E. coli*.** When P2 infects *E. coli* mutants temperature sensitive in the *dnaB* locus, no progeny phage are produced at 42 C, although they do appear at 30 C (Table 2). In addition, P2 DNA synthesis does not occur at 42 C in *ts-dnaB* strains, whereas it is normal at 30 C (D. Usher, unpublished data; D. Bowden, personal communication). Thus P2 requires the product of the *dnaB* gene for its DNA replication.

### P2 mutants with a relaxed requirement for

TABLE 1. Phage strains used

Designation	Pertinent Phenotype	Source or reference
P2	Wild type	1
P2 <i>vir</i> <sub>1</sub>	Unable to establish immunity	4
P2 <i>amB</i> <sub>213</sub>	Early mutant	26
P2 <i>amB</i> <sub>116</sub>	Early mutant	18
P2 <i>cox</i> <sub>4</sub> <i>amA</i> <sub>127</sub>	Excision deficient, early mutant	20
P2 <i>vir</i> <sub>24</sub> <i>tsB</i> <sub>40</sub>	Insensitive to immunity, early mutant	18
P2 <i>c</i> <sub>5</sub> <i>amB</i> <sub>116</sub>	Temperature-sensitive immunity, early mutant	18
P2 <i>vir</i> <sub>1</sub> <i>rlb</i> <sub>1</sub>	Increased ability to grow in <i>ts-dnaB</i> strains at intermediate temperatures	This study
P2 <i>vir</i> <sub>301</sub> <i>rlb</i> <sub>1</sub>	Insensitive to immunity, increased ability to grow in <i>ts-dnaB</i> strains at intermediate temperatures	This study

TABLE 2. Burst sizes<sup>a</sup>

Host strain and genotype	Genotype of infecting phage	Phage produced per infected cell at:		
		30 C	37 C	42 C
CRT266 <i>ts</i>	<i>rlb</i>	38	33	0.4
	+	30	4	0.3
CRT266R <i>ts</i> <sup>+</sup>	<i>rlb</i>	33	46	45
	+	34	41	42
LD312 <i>ts</i>	<i>rlb</i>	107	83	0.2
	+	91	10	0.1
H502 <i>ts</i> <sup>+</sup>	<i>rlb</i>	93	74	24
	+	84	78	37
FA22-1 <i>ts</i>	<i>rlb</i>	40	30	0.4
	+	32	4	0.2
K12SH28 <i>ts</i> <sup>+</sup>	<i>rlb</i>	ND <sup>b</sup>	42	ND
	+	ND	37	ND

<sup>a</sup> The experiments were performed as described. Bacteria were grown at 30 C and infected at the above indicated temperatures.

<sup>b</sup> ND, Not determined.

**the *dnaB* product.** The temperature dependence of P2 growth on *ts dnaB* strains can be tested by plating phage on a lawn of such strains, as long as the bacteria grow well enough to form a lawn. (This approach was suggested by C. Georgeopoulos.) At 36 to 37 C, strain CRT266 will form a lawn, but plates P2 very poorly (efficiency of plating,  $10^{-6}$ ). Mutants of P2 which grow well on *dnaB-ts* strains at 37 C have been selected by their ability to form plaques on CRT266 at 37 C. These mutants are termed *rlb*, for relaxed *dnaB* interaction or requirement. One such mutant, P2 *rlb*<sub>1</sub>, has been characterized further.

**P2 *rlb* phenotype.** When P2 *vir*<sub>1</sub> *rlb*<sub>1</sub> is grown in *dnaB* mutants at 37 C, the burst size is five- to eightfold larger than the burst produced by P2 *vir*<sub>1</sub> (Table 2). However, P2 *vir*<sub>1</sub> *rlb*<sub>1</sub> cannot grow at 42 C on any of the *dnaB* mutants tested. Growth is normal at 30 C. P2 *rlb*<sub>1</sub> will form plaques on CRT266 and LD312 at 37 C but not at 38 C, since these strains do not form good lawns at the higher temperature. FA22-1 will plaque P2*rlb*<sub>1</sub> at 34 C but not at 35 C. However, P2*rlb*<sub>1</sub> will produce a burst of 30 phage/cell at 37 C on this strain. This discrepancy has not been examined further.

**Dominance tests.** When P2 *vir*<sub>1</sub> and P2 *vir*<sub>1</sub> *rlb*<sub>1</sub> are grown together in *dnaB* strains at 37 C, bursts of phage are produced (Table 3). These bursts consist mostly of P2 *vir*<sub>1</sub> *rlb*<sub>1</sub>, whereas P2 *vir*<sub>1</sub> is produced at the same low level observed during single infections. Thus, the *rlb*<sub>1</sub> mutation is *cis* dominant.

TABLE 3. Dominance tests<sup>a</sup>

Host strain	Genotype	Infecting phage	Phage yield per infected cell and phenotype
FA22-1	<i>ts</i>	P2 <i>rlb</i> <sub>1</sub>	} mixed { 37 <i>rlb</i> <sub>1</sub> 6 +
FA22-1	<i>ts</i>	P2	
FA22-1	<i>ts</i>	P2 <i>rlb</i> <sub>1</sub>	} mixed { 51 <i>rlb</i> <sub>1</sub> 8 +
K12SH28	<i>ts</i> <sup>+</sup>	P2	
		P2 <i>rlb</i> <sub>1</sub>	} mixed { 50 <i>rlb</i> <sub>1</sub> 43 +
		P2	
CRT266	<i>ts</i>	P2 <i>rlb</i> <sub>1</sub>	} mixed { 10 <i>rlb</i> <sub>1</sub> 2 +
CRT266	<i>ts</i>	P2	
CRT266	<i>ts</i>	P2 <i>rlb</i> <sub>1</sub>	} mixed { 14 <i>rlb</i> <sub>1</sub> 3 +
CRT266R	<i>ts</i> <sup>+</sup>	P2	
		P2 <i>rlb</i> <sub>1</sub>	} mixed { 33 <i>rlb</i> <sub>1</sub> 27 +
		P2	

<sup>a</sup>The experiments were performed at 37 C as described. All phage carried the *vir*<sub>1</sub> mutation. Progeny phage were scored by plating on C-1055 at 37 C, picking plaques with sterile toothpicks into wells of broth plus chloroform, and replicating onto CRT266 at 37 C to test the phenotype. The values given in the first section of the table represent the average values obtained from two experiments.

**P2 and P2 *rlb* DNA synthesis in mitomycin C-pretreated cells.** The data presented in Table 2 indicate that some P2 DNA synthesis must be occurring upon infection of *dnaB* strains by P2 and P2 *rlb*<sub>1</sub> at 37 C, since phage bursts were observed. An analysis of P2 and P2 *rlb*<sub>1</sub> DNA synthesis in a *ts-dnaB* strain was made possible when Dumas and Miller (11) isolated a *ts-dnaB* derivative of a *uvrA* strain of *E. coli* C (LD312). Mitomycin C pretreatment of *uvrA* cells selectively suppresses host DNA synthesis while allowing P2 DNA synthesis to occur (11, 21).

P2 DNA synthesis in *ts-dnaB* and *ts*<sup>+</sup> strains was measured by the cumulative incorporation of tritium-labeled thymidine into trichloroacetic acid-insoluble material. Incorporation of label upon P2 *rlb*<sub>1</sub> infection of the *ts-dnaB* strain was approximately 50% of that found for the *ts*<sup>+</sup> strain, whereas incorporation of label upon P2 infection of the *ts-dnaB* strain was only 10% of that found for the *ts*<sup>+</sup> strain (Fig. 1). These results for the cumulative incorporation of label are consistent with those obtained from the biological assays (Table 2), i.e., fivefold higher incorporation of label for P2*rlb*<sub>1</sub>, compared with five- to eightfold higher burst sizes.

Dna synthesis in the *ts-dnaB* strain LD312 and the *ts*<sup>+</sup> strain H502 is essentially equivalent at 37 C (Fig. 1B). This indicates that 37 C is still permissive for *E. coli* DNA synthesis but

not for P2 DNA synthesis in LD312. This can be correlated with the use of LD312 as the plating culture for P2 at 37 C; the lawns grow well but wild-type P2 does not produce plaques. If the temperature is raised to 38 C, LD312 does not grow well.

**Mapping of *rlb*<sub>1</sub>.** Table 4 demonstrates that the *rlb*<sub>1</sub> mutation maps in the early region of the P2 genetic map, where the marker order (*vir*<sub>1</sub>, *c*<sub>5</sub>) (*vir*<sub>24</sub>, *cox*<sub>4</sub>) (*amB*<sub>116</sub>, *tsB*<sub>40</sub>) *amA*<sub>127</sub> has been previously established (15-18, 20). The mutations *vir*<sub>1</sub> and *c*<sub>5</sub> lie in the immunity gene C (5); *vir*<sub>24</sub> is a *cis*-dominant mutation, conferring insensitivity to immunity (18); *cox*<sub>4</sub> is a recessive mutation affecting spontaneous phage production from lysogens (20); genes A and B are needed for DNA replication (17, 18) and for late gene transcription (13). Included in the current mapping data are two crosses which locate *amB*<sub>213</sub> as the leftmost known marker in gene B

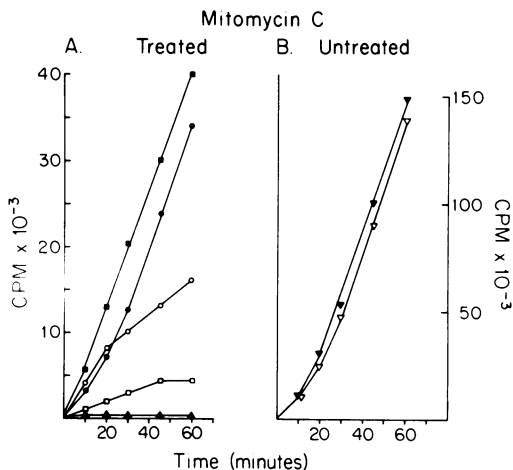


FIG. 1. (A) P2 DNA synthesis in mitomycin C-pretreated cells at 37 C. *E. coli* strains LD312 (*ts-dnaB*) and H502 (*ts*<sup>+</sup>) were grown in TPG-CAA plus thymine at 30 C and treated with mitomycin C (60 μg/ml) as described. Three aliquots (5 ml) were set up in the following manner: cells plus P2 *rlb*<sub>1</sub>, cells plus P2, cells alone. Both phage carried the *vir*<sub>1</sub> mutation. Multiplicity of infection was between 5 and 8. Phage were added after the mitomycin treatment, defined as *t* = 0. Five minutes later, 60 μCi of [<sup>3</sup>H]thymidine (specific activity, 5 Ci/mmol) was added to each aliquot. Incorporation of label into trichloroacetic acid-precipitable material was measured at 37 C as described. Open symbols refer to LD312; closed symbols refer to H502. Cells plus P2*rlb*<sub>1</sub> (O, ●); cells plus P2 (□, ■); uninfected cells (Δ, ▲). (B) Host DNA synthesis in mitomycin C-untreated cells at 37 C. LD312 and H502 were grown as in (A). [<sup>3</sup>H]thymidine (60 μCi) was added to 5-ml samples at *t* = 0, and incorporation of label into trichloroacetic acid-precipitable material was measured at 37 C as described. LD312 (▽), H502 (▼).

TABLE 4. Phage crosses<sup>a</sup>

Cross no.	Parental genotypes	Selection (% yield) <sup>b</sup>	Segregation of unselected markers
Order previously established: ( <i>vir</i> <sub>1</sub> , <i>c</i> <sub>5</sub> ) ( <i>vir</i> <sub>24</sub> , <i>cox</i> <sub>4</sub> ) ( <i>amB</i> <sub>116</sub> , <i>tsB</i> <sub>40</sub> ) <i>amA</i> <sub>127</sub>			
1	<i>c</i> <sub>5</sub> + <i>amB</i> <sub>116</sub> + <i>amB</i> <sub>213</sub> + Order deduced: <i>c</i> <sub>5</sub> <i>amB</i> <sub>213</sub> <i>amB</i> <sub>116</sub>	<i>am</i> <sup>+</sup> (0.030)	<i>c</i> <sub>5</sub> = 1,014 <i>c</i> <sup>+</sup> = 27
2	<i>vir</i> <sub>24</sub> + <i>tsB</i> <sub>40</sub> + <i>amB</i> <sub>213</sub> + Order deduced: <i>vir</i> <sub>24</sub> <i>amB</i> <sub>213</sub> <i>tsB</i> <sub>40</sub> Order overall: ( <i>vir</i> <sub>1</sub> , <i>c</i> <sub>5</sub> , <i>vir</i> <sub>24</sub> ) <i>amB</i> <sub>213</sub> ( <i>amB</i> <sub>116</sub> , <i>tsB</i> <sub>40</sub> )	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup> (0.056)	<i>vir</i> <sub>24</sub> = 623 <i>vir</i> <sup>+</sup> = 75
3	<i>vir</i> <sub>1</sub> <i>rlb</i> <sub>1</sub> + + + <i>amB</i> <sub>213</sub> Conclusion: <i>rlb</i> <sub>1</sub> is closer to <i>amB</i> <sub>213</sub> than to <i>vir</i> <sub>1</sub>	<i>am</i> <sup>+</sup> <i>vir</i> <sup>+</sup> (0.059)	<i>rlb</i> <sub>1</sub> = 39 + = 6
4	<i>vir</i> <sub>1</sub> <i>rlb</i> <sub>1</sub> + + + <i>amB</i> <sub>116</sub> Conclusion: <i>rlb</i> is located about midway between <i>vir</i> <sub>1</sub> and <i>amB</i> <sub>116</sub> Order deduced: <i>vir</i> <sub>1</sub> <i>rlb</i> <sub>1</sub> <i>amB</i> <sub>213</sub> <i>amB</i> <sub>116</sub>	<i>am</i> <sup>+</sup> <i>vir</i> <sup>+</sup> (0.023)	<i>rlb</i> <sub>1</sub> = 39 + = 26
5	<i>vir</i> <sub>1</sub> + <i>rlb</i> <sub>1</sub> + + <i>cox</i> <sub>4</sub> + <i>amA</i> <sub>127</sub>  Marker order deduced: <i>vir</i> <sub>1</sub> - <i>cox</i> <sub>4</sub> - <i>rlb</i> <sub>1</sub> - <i>amA</i> <sub>127</sub>	<i>am</i> <sup>+</sup> <i>vir</i> <sup>+</sup> (0.10)	<i>cox</i> <sub>4</sub> + = 95 <i>cox</i> <sub>4</sub> <i>rlb</i> <sub>1</sub> = 8 + <i>rlb</i> <sub>1</sub> = 10 + + = 1
6	<i>vir</i> <sub>301</sub> + <i>rlb</i> <sub>1</sub> + + <i>cox</i> <sub>4</sub> + <i>tsB</i> <sub>40</sub>  Marker order deduced: <i>vir</i> <sub>301</sub> - <i>cox</i> <sub>4</sub> - <i>rlb</i> <sub>1</sub> - <i>tsB</i> <sub>40</sub>	<i>ts</i> <sup>+</sup> <i>vir</i> <sup>+</sup> (0.082)	<i>cox</i> <sub>4</sub> + = 27 <i>cox</i> <sub>4</sub> <i>rlb</i> <sub>1</sub> = 16 + <i>rlb</i> <sub>1</sub> = 14 + + = 4
Overall order: ( <i>vir</i> <sub>1</sub> , <i>c</i> <sub>5</sub> ), ( <i>vir</i> <sub>301</sub> , <i>vir</i> <sub>24</sub> ) <i>cox</i> <sub>4</sub> <i>rlb</i> <sub>1</sub> <i>amB</i> <sub>213</sub> ( <i>amB</i> <sub>116</sub> , <i>tsB</i> <sub>40</sub> ) <i>amA</i> <sub>127</sub>			

<sup>a</sup> Crosses involving P2 amber mutants were performed in C-1757 at 37 C, and those involving P2 *ts* mutants were performed in C-1a at 30 C. *Vir*<sup>+</sup> recombinants were selected visually as turbid plaques, reisolated as single plaques, and tested for other markers. The marker *cox*<sub>4</sub> was scored as the inability to release phage spontaneously after lysogenization of *E. coli* C-1a (20). The *rlb* phenotype was tested on CRT266 at 37 C.

<sup>b</sup> Percent of yield is the observed frequency of the selected recombinant type in percent of total yield.

(Table 4, crosses 1 and 2). Cross 3 shows that the *rlb*<sub>1</sub> mutation lies nearer to *amB*<sub>213</sub> than to *vir*<sub>1</sub>. Cross 4 shows that *rlb*<sub>1</sub> is located about midway between *vir*<sub>1</sub> and *amB*<sub>116</sub>. These data suggest the order *vir*<sub>1</sub> *rlb*<sub>1</sub> *amB*<sub>213</sub> *amB*<sub>116</sub>. The *rlb*<sub>1</sub> mutation can be ordered relative to the *cox*<sub>4</sub> mutation by a four-factor cross (cross 5). Recombination is selected between the immunity gene and gene A, and the *cox*<sub>4</sub> and *rlb*<sub>1</sub> markers are scored. The least frequent recombinant class (*cox*<sup>+</sup> *rlb*<sup>+</sup>) must derive from the most-complicated recombinational event, and thus the order must be *vir*<sub>1</sub> *cox*<sub>4</sub> *rlb*<sub>1</sub> *amA*<sub>127</sub>. The

order of *cox*<sub>4</sub> and the immunity-insensitive mutation *vir*<sub>301</sub> can be deduced from analysis of the similar cross (cross 6), which selects for recombination between *vir*<sub>301</sub> and a *ts* mutant in gene B. Given the order of *rlb*<sub>1</sub> and *cox*<sub>4</sub> deduced from cross 5, *vir*<sub>301</sub> must be to the left of *cox*<sub>4</sub>. Thus, the most likely order for markers in the early region is C-*vir*<sub>301</sub>-*cox*<sub>4</sub>-*rlb*<sub>1</sub>-B-A.

## DISCUSSION

For normal DNA replication, phage P2 requires the products of *E. coli* genes *rep* (6), *dnaE* pol III), and *dnaG* (D. Bowden, personal

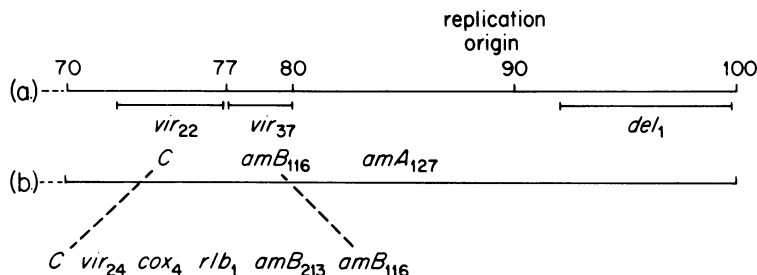


FIG. 2. Physical and genetic map of the P2 early region. (a) Physical map of the P2 early region derived from Bertani (2), Bertani and Bertani (3), Schnös and Inman (24), and Chatteraj and Inman (7, 8). (b) Genetic map of the phage P2 early region derived from Lindahl (15-18), Lindahl and Sunshine (20), and from the data in Table 4. Map distances are not accurately shown.

*dnaE* (pol III), and *dnaG* (D. Bowden, personal communication), as well as the products of phage genes A and B (17, 21). This paper implicates the *dnaB* gene product as an essential element for P2 DNA replication, since P2 cannot produce progeny on *dnaB* mutant hosts at nonpermissive temperatures (Table 2). However, phage mutants can be isolated (P2 *rlb*) which produce five- to eightfold more progeny than P2<sup>+</sup> on *dnaB* mutant host strains at intermediate temperatures (Table 2). The P2 *rlb* mutation does not obviate the requirement for the *dnaB* product, but does alter the quantitative or qualitative nature of the requirement.

The P2 *rlb* mutation which we have characterized is *cis* dominant. Since it does not map in gene A, whose product is *cis* acting, the *rlb* mutation may represent either a site or a second gene with a *cis*-acting product. The *rlb* mutation maps in genetic crosses to the left of several mutants in P2 gene B, but to the right of the *cox4* mutation (Table 4; Fig. 2). P2 gene B, unlike gene A, is not *cis* acting. This would tend to rule out the possibility that *rlb* is in gene B. However, it still remains that *rlb* might be in the promoter to gene B (and gene A), since *rlb* is *cis* dominant as are other known promoter mutations.

If we assume that the genetic and physical maps of P2 are colinear in the region between immunity (gene C) and *amB*<sub>116</sub>, then the *rlb* mutation can be located in a small region of the P2 genome. We assume that the virulent deletion mutation *vir*<sub>22</sub> (Fig. 2) covers the site for the virulent mutation *vir*<sub>301</sub> as it does for the virulent mutation *vir*<sub>24</sub> (2). Second, we accept that the virulent duplication mutation *vir*<sub>37</sub> covers the *amB*<sub>116</sub> mutation (3). If these assumptions are true, then the *rlb* mutation must lie between the limits of these two chromosomal aberrations (72 to 80% of the DNA), since *rlb* maps genetically between *vir*<sub>301</sub> and *amB*<sub>116</sub>.

The first conclusion to be drawn from this

mapping data is that *rlb* cannot be a mutation of the replication origin, which lies at 89% on the wild-type P2 genome (24; Fig. 2). What then might be the role of the *rlb* mutation? Since the promoter for early P2 transcription is thought to lie in the same region as the *rlb* mutation (3, 18) and early P2 transcription proceeds from left to right (13, 22), the *rlb* mutation might affect the level of transcription over the replication origin (10), and more or less transcription might be required in the presence of limiting or defective *dnaB* product.

Alternatively, the *rlb* mutation might simply affect the amount of A gene product synthesized. Since the A gene product acts only in *cis*, the *cis* dominance of the *rlb* mutation would be explained in this case.

Another interpretation is that *rlb* DNA can utilize or react with an altered structural component of the host cell's replication apparatus. Since the P2 *rlb* mutation has been found to be *cis* dominant, it may represent a site which interacts with the *dnaB* gene product of the host. At the intermediate temperature, 37 C, the *dnaB* protein might only have a minor alteration in its structure. The *rlb* mutation could then be an alteration of the P2 site which can recognize a partially changed *dnaB* protein, allowing P2 DNA replication to occur. At 42 C, the *dnaB* protein would be grossly changed such that it could not interact with P2 *rlb* DNA.

#### ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-04043, AI-05367, and AI-08722 from the National Institute of Allergy and Infectious Diseases, by training grants CA-5028 from the National Cancer Institute and 52-2107-6457 from the National Institute of Allergy and Infectious Diseases, and by American Cancer Society Institutional Research grant 1N-21M to the University of Southern California School of Medicine.

One of us (M. S.) is grateful to M. Lieb and I. Gordon for the hospitality of their facilities where part of this work was performed. We thank E. W. Six and M. Feiss for many valuable discussions. We thank Don Bowden for bringing

strain LD312 to our attention and for communicating his data to us. We thank L. Dumas for providing strain LD312 prior to publication.

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