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Isolation and Preliminary Characterization of *Escherichia coli* Mutants Resistant to Lethal Action of the Bacteriophage λ *P* Gene

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Both spontaneous and NTG-induced mutants of *Escherichia coli* 594 insensitive to the lethal action of λ *P* gene were isolated and called *rpl* (*r*esistant to *P* *l*ethality). These mutants were of two types, showing different phenotypes. On type I *rpl* mutants, λ*c*I⁻ and λ*v*1*v*3 did not plate, while λ*vir*, λ*c*I⁻ *c*17, λ*imm*434, and λ*imm*21 did; plasmid pMR45 carrying the λ*P* gene could not complement λ*imm*21*P*⁻ phage in type I mutants. On the other hand, the type II *rpl* mutants support the growth of all the above phages including λ*c*I⁻. Neither type of *rpl* mutation affects growth of the bacteria. © 1991 Academic Press, Inc.

In a preceding paper, we showed that elevated levels of functional product of bacteriophage λ replication gene *P* are lethal to *Escherichia coli* even in the absence of DNA replication from the phage origin (*1*). To understand the mechanism of this lethal interaction of the *P* gene product with its host, we isolated *E. coli* mutants resistant to λ *P* lethality.

Both spontaneous and chemically induced mutants of *E. coli* 594 were isolated as described in Table 1. λ*c*I⁻ could not plate on all the spontaneous mutants while the mutants isolated from a mutagenized culture contained this type (I) and also a second type (II) on which λ*c*I⁻ could plate. Both these types were called *rpl* mutants (*rpl* = *r*esistant to λ *P* *l*ethality). One, *rpl*-1, from the former set and three, *rpl*-7, *rpl*-8, and *rpl*-9, from the latter set were challenged with λ *P*-carrying plasmid pMR45 which could provide a lethal level of *P* in wild-type 594. The results presented in Table 1 clearly show that all the mutants tested could survive transformation with this plasmid.

In Table 2 we present data on the plating efficiencies of different λ phages. The results indicate that *rpl*-1 and *rpl*-7 represent type I mutants on which λ*c*I⁻ did not plate at all (e.o.p. less than 10^{-8}) while λ*vir* and λ*c*I⁻*c*17 plated with efficiencies of 0.9 and 1.0 and 0.25 and 0.34, respectively, producing smaller plaques relative to those on 594. λ*v*1*v*3, having constitutive mutations only in *o*R, plated on both the type I *rpl* bacteria and 594(λ⁺) with the same low efficiency of 10^{-5}. The mutants *rpl*-8 and *rpl*-9 represent type II, on which λ*c*I⁻ plated with efficiencies of 0.24 and 0.34, and λ*vir*, λ*v*1*v*3, and λ*c*I⁻*c*17 plated with efficiencies ranging from 0.4 to 1.0, producing plaques having sizes comparable to those on 594. λ*imm*434 and λ*imm*21 phages plated on both the type I and the type II mutants with 100% efficiency. The average burst sizes of λ*c*I⁻ in 594, *rpl*-1, *rpl*-7, and *rpl*-8, determined under identical conditions, were 172, 1.5, 2.1, and, 85.7 respectively. These results support the plating data of λ*c*I⁻ phage on the mutants (Table 2).

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TABLE 1

SURVIVAL OF 594, 594(λ⁺), AND *rpl* MUTANTS AFTER TRANSFORMATION WITH λ *P* PLASMID pMR45

Bacteria[a]	Transformation efficiency (No. of transformants/μg DNA)
594	<10
594(λ⁺)	6.37×10^4
rpl-1	4.00×10^4
rpl-7	5.37×10^4
rpl-8	5.76×10^4
rpl-9	6.05×10^4

[a] The spontaneously occurring *rpl* mutants of *E. coli* were isolated by transforming 594 with around 0.5 μg of plasmid pMR45 DNA by the procedure described (*1*) and selecting the survivors on ampicillin plates after 20 hr at 32° (the plasmid pMR45 carries the *P* gene of λ, and the wild-type bacteria transformed with this plasmid do not survive). The λ *P*-resistant host mutants were also isolated by mutagenizing with *N'*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the procedure of Miller (*2*). The mutagenized bacteria at around 1% survival were grown in Luria broth to saturation at 32°. These bacteria were then transformed with pMR45 using around 40 ng DNA by the procedure described (*1*). The transformants surviving on ampicillin plates were picked. Around six healthy colonies from each of the above two sets were purified. Plasmids were cured from them by growing in broth in the absence of ampicillin for 3–4 days at 32°. These were plated in the absence of the drug and the plasmid-cured bacteria were isolated by selecting the ampicillin-sensitive colonies by replica plating.

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TABLE 2

EFFICIENCIES OF PLATING OF DIFFERENT λ AND ITS HETEROIMMUNE DERIVATIVES ON 594, 594(λ⁺), AND DIFFERENT *rpl* MUTANTS[a]

Phage[b]	EOP on[c]					
	594	594(λ⁺)	*rpl*-1	*rpl*-7	*rpl*-8	*rpl*-9
λ*cI*⁻	1.00	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	0.24	0.34
λ*vir*	1.00	1.00	0.90[d]	1.00[d]	1.06	0.95
λ*v*1*v*3	1.00	10^{-5}	10^{-5}	10^{-5}	0.40	0.50
λ*cI*⁻ *c*17	1.00	0.75[d]	0.25[d]	0.44[d]	0.98	1.02
λ*imm*434	1.00	1.09	1.04	0.97	0.96	0.90
λ*imm*21	1.00	0.90	1.05	1.10	1.02	0.98

[a] Bacteria were grown in TBM (Ref. (*3*)) to A_{590} of 1.00, and the phages were assayed at suitable dilutions.
[b] λ*cI*⁻, λ*vir*, λ*v*1*v*3, λ*imm*434, and λ*imm*21 were obtained from Dr. M. Lieb, and λ*cI*⁻*c*17 was from Dr. S. Adhya.
[c] EOP on all other bacteria were determined relative to 594.
[d] Plaques of λ*vir* were relatively smaller while those of λ*cI*⁻ *c*17 were very tiny on *rpl*-1 and *rpl*-7.

From the plating data of λ and its heteroimmune derivatives, one can speculate on the mechanisms of resistance to λ*P* lethality due to the two types of *rpl* mutations. Type I mutations, *rpl*-1 and *rpl*-7, affected the growth of wild-type λ but not that of λ*vir*. This suggests that inhibition is imposed on the expression of genes from *p*R of wild-type λ by the type I *rpl* mutation which could not be seen with λ*vir* because the latter has constitutive mutations in *o*R (and *o*L). Also, in the case of λ*cI*⁻*c*17, the *c*17 mutation, which creates a new rightward promoter by a 9-bp duplication around the *pre* site between the C-terminus of *cro* and N-terminus of *c*II, helps expression of the downstream genes even when the *o*R*p*R is blocked by repressor (*4–6*). So, λ*cI*⁻ *c*17 could grow even when the expression of genes from *p*R is inhibited by type I *rpl* mutation. These results suggest that the inhibition of expression of *P* in type I *rpl* host is specific for the wild-type *o*R but not for *o*R having constitutive mutations.

The fact that λ*vir* but not λ*v*1*v*3 plate on type I *rpl* mutants suggests that either the expression from wild-type *o*L*p*L is also inhibited in these bacteria or the mutant protein does not show such inhibition but the constitutive expression of *cro* to a higher level due to *v*1*v*3 mutations in *o*R leads to quick blockage of wild type *o*L*p*L, and so, the growth of the latter phage is inhibited.

The type II *rpl* mutants allow the growth of λ*cI*⁻ (Table 2). This suggests that the expression from both *p*R and *p*L of wild-type λ is not suboptimal with respect to λ DNA replication in this mutant host. So, the resistance to λ *P* gene lethality by type II mutants may be due to a mechanism in which the P protein fails to interact with a mutated target component of the host. Since wild-type λ could grow in a type II mutant, and all three types of *gro*P mutant *E. coli* (*7–9*) are equally susceptible to killing by P (*1*), it may be assumed that the lethal action of this replication protein of λ is not dependent on its interaction with any of the known host functions that are involved in λ DNA replication (*10–12*).

It was further observed that 594 and both types of *rpl* mutant grew with identical rates in nutrient broth as well as in minimal medium at either 32° or 42°, and the cell sizes of all the mutants were also comparable with that of 594 (data not shown). These results suggest that the *rpl* mutations do not affect an essential gene of the host.

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