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Bacterial Mutants Defective in Plasmid Formation: Requirement for the *lon*$^+$ Allele

(transduction/phage P1/*E. coli*/lysogeny/UV reduce sensitivity)

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ABSTRACT **Bacterial mutants defective in plasmid formation were selected for their inability to be transduced to chloramphenicol resistance by bacteriophage P1CM. The mutants isolated are indistinguishable from a *lon* mutant strain. Both the *lon* strain and the mutants isolated here show very poor lysogenization by P1 and very low transduction to Gal$^+$ by the plasmid formation of the λ*gal*$_8$-*Nam7am53cI*$_{857}$. The *lon*$^+$ gene function of the host bacteria is indispensable for plasmid formation, even though P1 and λ$^+$ can grow normally on the *lon* strains and λ$^+$ can be integrated normally in *lon* as well as in the *lon*$^+$ bacteria. Phage mutants that can persist as plasmids in *lon* strains were isolated from P1CM. The function of the *lon*$^+$ gene is discussed with regard to plasmid formation.**

Bacterial plasmids (1) are favorable tools for the study of the mechanism and regulation of DNA replication, because they are smaller than the host chromosome and are not essential for bacterial growth, and because their genetic and chemical structures have been well studied. Recently it was reported that a mutant of phage λ defective in gene *N* (2, 3), a defective virulent mutant of λ (4), and prophage P1 (5) can persist in *Escherichia coli* as plasmids. These bacterial plasmids appear to replicate independently of the host chromosome, but in approximate synchrony with bacterial cell division. In order to study the bacterial functions involved in the establishment and replication of plasmids, I have isolated bacterial mutants defective in lysogenization by P1. The mutant strains turn out also to be defective in plasmid formation by the amber *N* mutant of λ and to carry a mutation in the gene known as *lon* (6–8), which has pleiotropic effects on UV sensitivity, septum formation, and the regulation of capsular polysaccharide production.

MATERIALS AND METHODS

Bacterial and bacteriophage strains

The bacterial strains used in this study are described in Table 1. Phage P1 was from the collection of S. E. Luria. The clear mutant P1*c*13 was obtained from J. R. Scott (9), who also provided P1CM (10), the recombinant of P1 and the chloramphenicol-resistance (CM^R) marker of an R factor. The derivatives of phage λ were obtained from E. R. Signer.

Media

LB broth and agar (11) were used for bacterial cultures and the experiments with P1. 27 μg of chloramphenicol per ml was added to LB agar to select the bacteria lysogenic for P1CM. λ-broth, agar, and soft agar (11) were used in the experiments with λ. Minimal medium (11) in which glucose was replaced by galactose (supplemented by 20 μg of amino acids and 5 μg of adenosine per ml as needed) was used for the transduction of the galactose marker (Gal$^+$) by λ*gal*$_8$ *Nam7am53cI*$_{857}$ (hereafter abbreviated as λ*gal*N$^-$) (2). SM buffer (2) was used for dilution of λ stocks. EMB-latrose agar, containing 750 μg of dihydrostreptomycin per ml, was used as a selective medium for bacteria carrying the F13 sex factor (12).

Isolation of bacterial mutants defective in lysogenization by P1

AB1157 or M72*gal* were used as parental strains. Logarithmically growing cultures were treated with 300 μg/ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine) for 10 min by the procedure of Adelberg *et al.* (19). After growth for several generations, the cultures were infected with P1CM at a multiplicity of 10 and incubated for several generations at 42°C. Chloramphenicol-sensitive (CM^S) bacteria in the cultures were selected by penicillin screening as described by Gorini *et al.* (20). Survivors were plated on LB agar with 10^6 plaque-forming units (PFU)/plate of P1*c*13 phage (9). After incubation for 20 hr at 37°C, nibbled colonies were picked and purified. The CM^S and P1-sensitive (P1^S) clones were tested for their ability to serve as recipients in transduction of CM^R by P1CM (see below).

Lysogenization by P1

To about 5×10^7 bacteria in 0.1 ml of LB broth was added 1 drop of 5×10^{-2} M $CaCl_2$ and P1 phage, to a multiplicity of 10. After adsorption at 37°C for 20 min, the infected cultures were diluted 1000-fold with warmed LB broth. Then, after overnight incubation at 37°C, the bacteria were plated on LB agar with 10^6 PFU of P1*c*13 per plate. After 20 hr incubation at 37°C, the ratio of non-nibbled to nibbled colonies was determined.

Transduction of CM^R by P1CM

Bacteria were infected with P1CM at a multiplicity of 0.05 and the cultures were diluted with LB broth and incubated over-night, and the numbers of CM^R bacteria were determined.

Transduction of galactose utilization (Gal$^+$) by λgalN$^-$

The methods were described by E. Signer (2).

Isolation of P1CM mutants that can lysogenize lon as well as lon$^+$ bacteria

P1CM was adsorbed to K175 at a multiplicity of 5. After adsorption, the P1CM-infected bacteria were sus-

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TABLE 1. *Bacterial strains*

Strain	*Lon* character	Markers	Source
AB1157	+	F⁻, *thr, pro*A, *his, thi, arg*E, *lac*Y, *gal, xyl, ara, man, tsx, str, su*⁺	A. J. Clark
A205	−	Derivative of AB1157	
A240	−	Derivative of AB1157	
AB1899	−	Derivative of AB1157	H. I. Adler (6)
AB2463	+	*rec*A13, derivative of AB1157	P. Howard-Flanders (13)
AB1866	+	*uvr*A6, derivative of AB1157	P. Howard-Flanders (13)
M72*gal*⁻	+	F⁻, lac_{am}Y14, *trp, str, gal*	E. R. Signer
No. 2035	−	Derivative of M72*gal*⁻	
MC100	+	*leu, pro*C, *pur*E, *trp, lac, gal, str*	A. Markovitz (14)
MC102	−	*pro*⁺, *lon* transductant of MC100, *cap*R9	A. Markovitz (14)
MC103	−	*pro*⁺, *lon* transductant of MC100, *cap*R66	A. Markovitz (14)
S43-2	−	*pro*⁺, *lon* transductant of MC100, *cap*R6	A. Markovitz (14)
A205MC	−	*pro*⁺, *lon* transductant of MC100 from A205	
A240MC	−	*pro*⁺, *lon* transductant of MC100 from A240	
2e10c	+	F⁻, *lac, thi, thr, str, su*II⁺	Walker and Pardee (15)
AX14	−	Derivative of 2e10c	Walker and Pardee (15)
P678	+	F⁻, *thr, leu, thi, str*, λ^R	H. I. Adler *et al.* (16)
P678-A4	−	Derivative of P678	H. I. Adler *et al.* (16)
P678-7	+	*min*, derivative of P678	H. I. Adler *et al.* (16)
P678-56	+	Amorphous, derivative of P678	H. I. Adler *et al.* (16)
lon-min	−	*min*, derivative of P678	H. I. Adler
x984	+	*min*A, *min*B, F⁻, *pur*E, *pxd, his, str, xyl, ilv, met*	R. Curtiss, III
W3747	+	*met, thi*, (F13⁺; *lac* ~ *pur*E)	Y. Hirota (12)
200PS	+	(F′-*lac*⁺)	F. Jacob
P4x	+	*rec*A, *spc*, Hfr (←*pro*A, *thr, leu, arg*E)	I. M. Hertman (18)
φ1	+	*su*II⁺, indicator strain for λ amber mutants	E. R. Signer
WD5021	+	*su*⁻, indicator strain for λ⁺	E. R. Signer
K175	+	*su*⁺, indicator strain for P1	J. R. Scott[9]

pended in buffer, treated with 100 μg/ml of nitrosoguanidine (19), and incubated for 100 min in LB broth at 37°C. Chloroform was added and the lysates were plated on a *lon* strain, AX14. Bacteria were picked from the center of the plaques and streaked on plates of LB agar containing chloramphenicol (10). The stable lysogens of P1CM can grow on these plates. Free phages were isolated from the stable lysogens, and their ability to transduce CM^R to AX14, compared with a *lon*⁺ strain, was determined.

RESULTS

Lysogenization of the bacterial mutants by P1 and P1CM

Three mutant bacterial strains, A205, A240, and #2035, were isolated on the basis of their very poor lysogenization by P1 or P1CM, though P1 was able to grow normally on them (Table 2). The P1 plaques and spot areas of lysis on plates of these mutants were much clearer than the parental strains. These mutants formed mucoid colonies on the minimal medium.

TABLE 2. *Lysogenization by P1 and P1CM and the plating efficiency of P1*

Strain	Lysogenization by P1*	CM^R transductants by P1CM per ml	Plating efficiency of P1
AB1157	976/1028	2.1×10^6	2.7×10^{10}
A205	0/168	1.8×10^4	2.0×10^{10}
A240	0/142	2.3×10^5	2.3×10^{10}
M72 *gal*⁻	934/982	9.8×10^6	3.4×10^{10}
No. 2035	0/151	5.7×10^4	2.9×10^{10}

* Non-nibbled colonies as a fraction of total colonies.

Transduction of the mutants to Gal⁺ by λ*gal*N⁻

Prophage P1 is a plasmid that replicates independently of the host chromosome (5). Transduction of the mutants to Gal⁺ by a λGal *sus*N mutant, which in su⁻ bacteria gives rise to a plasmid form (2), was studied as another instance of plasmid formation. The mutant used was λ*gal*N⁻, which has a temperature-sensitive repressor. Because AB1157 and its derivatives are su⁺, the mutations of A205 and A240 were transferred into strain MC100 su⁻ by cotransduction with *pro*C⁺

TABLE 3. *Gal⁺ transduction by λgalN⁻ and the plating efficiency of λ*

Strain	Gal⁺ transductants per 5×10^7 PFU of λ*gal*N⁻		Plating efficiency of λ⁺
	At 40°C	At 32°C	
M72 *gal*	2.5×10^7 (1.0)	3.2×10^7 (1.0)	7.6×10^9
No. 2035	4.7×10^4 (0.0019)	2.9×10^5 (0.0091)	8.1×10^9
MC100	1.7×10^7 (1.0)	2.0×10^7 (1.0)	6.9×10^9
A205MC	6.2×10^4 (0.0036)	6.1×10^4 (0.0030)	6.6×10^9
A240MC	9.1×10^4 (0.0054)	1.3×10^5 (0.0063)	5.3×10^9

The numbers in parentheses are the relative numbers of the Gal⁺ transductants in the mutant strains compared to the parental strains.

TABLE 4. *Stability of the Gal⁺ transductants by λgalN⁻*

Strain	Unstable/stable	
	At 40°C	At 32°C
M72*gal*	82/2	84/3
No. 2035	11/74	2/80
MC100	80/6	76/0
A205MC	9/82	1/67
A240MC	16/77	0/54

(see below). These derivatives were used in transduction tests. The results (Table 3) completely paralleled those of the lysogenization with P1 and P1CM, i.e., the frequency of transduction to Gal⁺ by λ*gal*N⁻ was markedly reduced in the mutants, whereas λ⁺, which produces lysogenization by integration, formed normal, turbid plaques with the same efficiency on the mutants as on the parental strains.

The Gal⁺ transductants by λ*gal*N⁻ are known to segregate Gal⁻ subclones at a rather high rate (2, 3). The stability of the Gal⁺ marker of the transductants of the mutants was determined by repeated reisolations (Table 4). Most Gal⁺ transductants of the parental strains were unstable; the majority of the rare Gal⁺ transductants of the mutants were rather stable. This implies that stable integration of the Gal⁺ marker by λ*gal*N⁻ into the parent and mutant strains may take place at equal (low) frequencies.

The frequency of formation of stable Gal⁺ transductants of parent and mutant strains by λ*gal*N⁻ was determined. Gal⁺ transductants stable at 32°C were found with equal frequencies in parent and mutant strains.

Other properties of the mutants

The UV sensitivity of the mutants was determined on LB agar plates and compared with that of the parental strains by the methods of Howard-Flanders *et al.* (13). As shown in Table 5, the mutants were much more sensitive to UV irradiation.

The Rec function of the mutants was compared with those of a *rec*⁺ and a *rec*A strain by the following four tests:

(i) Transduction of host chromosomal markers by P1;
(ii) Mating with Hfr P4x;
(iii) Production of *sus*⁺ recombinants in a cross of λ*int6red3 sus*N7N53cI_{857} × λ*int6red3susJ6c*I_{857} (which requires the host cell recombination function).
(iv) UV induction of λ⁺-lysogenic mutant strains.

TABLE 5. *Sensitivity to UV irradiation*

Strain	Surviving fraction after UV irradiation for	
	15 sec	30 sec
AB1157	0.99	0.78
A205	2.5×10^{-3}	5.2×10^{-4}
A240	3.3×10^{-2}	6.2×10^{-4}
M72*gal*	1.02	0.95
No. 2035	1.8×10^{-1}	7.8×10^{-2}
AB1886*	1.3×10^{-3}	9.2×10^{-5}
AB2463†	6.3×10^{-4}	8.2×10^{-5}

* AB1886 is *uvr*A.
† AB2463 is *rec*A.

TABLE 6. *Recombination function*

(*a*) Transduction of chromosomal markers by P1

Strain	Transductants per 10^8 PFU of P1		
	*pro*A⁺	*his*⁺	*arg*E⁺
AB1157	308	428	696
A205	255	365	330
A240	286	305	320
AB2463 (*rec*A)	0	1	0

(*b*) Recombination with Hfr P4x *rec*A*

Strain	Recombinants per 5×10^7 Hfr bacteria		
	*pro*A⁺	*thr*⁺	*arg*E⁺
AB1157	1.9×10^6	3.2×10^5	1.2×10^5
A205	7.2×10^5	1.3×10^5	2.8×10^4
A240	7.0×10^5	2.8×10^4	2.8×10^4
AB2463 (*rec*A)	$<1 \times 10^2$	$<1 \times 10^2$	$<1 \times 10^2$

(*c*) Cross λ*int6red3sus*N7N53cI_{857} × λ*int6red3sus*J6cI_{857}

Strain	Percent λ*sus*⁺ in the phage progeny
AB1157	6.4
A205	4.9
A240	3.2
AB2463 (*rec*A)	<0.01

* 5×10^7 Hfr bacteria were mixed with 3×10^8 recipients for 60 min at 37°C.

The results of experiments (i), (ii), and (iii), shown in Table 6, are closely similar for the mutants and the *rec*⁺ control. The prophage λ⁺ in the mutants was induced as efficiently by UV as in the parental strains. Therefore, the mutants as well as the parental strains appear to have a normal Rec⁺ function.

Host-cell reactivation (*hcr*) of UV-irradiated phage T1 was determined by the methods of Howard-Flanders *et al.* (13). The results (Table 7) show that the *hcr* function of the mutants is about the same as that of the parental strains.

The morphology of cells in cultures of the mutants A205, A240, and no. 2035 was examined under a phase-contrast microscope. The cells of mutant bacteria made markedly long snake forms; the cells of the parental strains were normal short rods.

The F13 sex factor, which is known to carry the wild-type alleles of the *lon* and *pro*C genes, was transferred from strain W3747 to the mutant strains A205, A240, and no. 2035 as efficiently as to the parental strains AB1157 and M72*gal*,

TABLE 7. *Host-cell reactivation of UV-irradiated phage T1*

On strain	T1 titer (PFU)		
	UV dose, seconds		
	0	60	120
AB1157	1.5×10^8	1.9×10^7	6.8×10^6
A205	9.9×10^7	1.1×10^7	6.1×10^6
A240	1.1×10^8	2.0×10^7	6.3×10^6
AB1886 (*uvr*A)	1.7×10^8	9.8×10^5	1.1×10^4

TABLE 8. *Plasmid formation in the mutants carrying F13*

On strain	CM^R transductants per ml	Gal$^+$ transductants* per 5×10^7 PFU $\lambda gal N^-$
M72*gal*	3.0×10^7	1.5×10^7
M72*gal* (F13)	2.8×10^7	2.8×10^6
No. 2035	7.2×10^3	5.0×10^3
No. 2035(F13)	9.8×10^6	3.8×10^6
MC100	4.3×10^7	1.1×10^7
MC100(F13)	4.6×10^7	1.8×10^6
A205MC	4.0×10^5	5.7×10^4
A205MC(F13)	2.7×10^7	5.6×10^6
A240MC	8.9×10^5	5.0×10^4
A240MC(F13)	3.7×10^7	6.7×10^6

* Gal$^+$ transductants were scored at 40°C.

and persisted with comparable stability in these strains. The mutant strains carrying F13 showed the same high transduction of CM^R or Gal$^+$ by P1CM or $\lambda gal N^-$ as parental strains with or without F13 (Table 8).

Mapping of the mutation sites

All the above properties of the mutants are the same as those of a known *lon* strain, which was found to be cotransduced with *pro*C by P1. The results of transduction tests shown in Table 9 indicate that the mutation sites of A205, A240, and no. 2035, like that of *lon*, are located close to *pro*C.

UV-resistant revertants were isolated from A205, A240, and no. 2035 by selection with a small dose of UV radiation. All UV-resistant mutants isolated (26 from A205, 18 from A240, and 38 from no. 2035) were also revertant with respect to snake formation, colony morphology, CM^R transduction by P1CM, and Gal$^+$ transduction by $\lambda gal N^-$. It appears, therefore, that in the mutant strains the defect in transduction of CM^R and Gal$^+$ by P1CM and $\lambda gal N^-$ is controlled by a single gene mutation.

Plasmid formation by P1CM and $\lambda gal N^-$ was studied on known *lon* strains. As shown in Table 10, all *lon* strains tested were poorly transduced for CM^R and Gal$^+$ by P1CM and $\lambda gal N^-$, respectively.

Mutants of P1CM that lysogenize the *lon* strain at high frequency

From P1CM, mutants called P1CM*p* were isolated that can lysogenize in the *lon* strain as well as in the *lon*$^+$ strain. The CM^R transduction by the P1CM*p* mutants on *lon* strain AX14 is shown in Table 11. P1CM*p* transduces CM^R to the *lon* strain at almost the same high frequency as to the *lon*$^+$ bacteria. On the *lon* indicator, the mutants produce plaques as turbid as those on the *lon*$^+$ bacteria.

TABLE 9. *Cotransduction of the mutation sites with the proC marker by P1*

Donor	Recipient	No. of *pro*C$^+$ transductants tested	No. of clones poorly transduced by P1CM
A205	MC100	285	31 (10.9%)
A240	MC100	177	21 (11.9%)
No. 2035	MC100	198	26 (13.1%)
AX14	MC100	153	20 (13.0%)

TABLE 10. *Plasmid formation in the lon strains*

Strain	*lon*	Other characters	CM^R transductants	Gal$^+$ transductants per 5×10^7 PFU $\lambda gal N^-$
AB1157	+		4.6×10^7	2.8×10^7*
AB1899	−		1.8×10^5	8.7×10^3*
AB2463	+	*rec*A	9.8×10^6†	
AB1866	+	*uvr*A	3.2×10^7	
MC100	+		6.2×10^7	2.5×10^7
MC102	−		1.4×10^6	1.7×10^4
MC103	−		5.2×10^5	1.0×10^4
S43-2	−		5.0×10^6	
2e01	+	*min*	7.3×10^6	1.7×10^7*
AX14	−		1.0×10^3	3.2×10^3*
P678	+		6.3×10^7	
P678-A4	−		1.8×10^4	
P678-7	+	*min*	1.3×10^7	
P678-56	+	*amph*	3.8×10^7	
lon-mini	−	*min*	1.1×10^3	
x984	+	*min*A *min*B	1.9×10^7	1.2×10^7*

* Gal$^+$ transduction by $\lambda gal N^-$ was titrated on strain MC100 that had previously received the mutation site by P1 cotransduction with the *pro*C marker.

† The lysogenization of P1CM, scored by CM^R transduction, is markedly suppressed in the *rec* bacteria (data to be published). The growth of P1 is also restricted in the *rec* strain (11). These evidences are specific for P1. $\lambda gal N^-$ can form plasmid in the *rec* host bacteria as well as in the *rec*$^+$ strain (2).

DISCUSSION

The present results show that *lon* mutants, as well as the strains isolated as defective in lysogenization by P1CM, do not support efficient plasmid formation by P1 and $\lambda gal N^-$. They do, however, support the growth of both phage P1 and λ. This indicates that in the establishment or maintenance of plasmids there is a process common to P1 and $\lambda gal N^-$ that requires the function of the host gene *lon*$^+$. The requirement for *lon*$^+$ is specific for P1 and $\lambda gal N^-$ plasmids, since F and R factors, as well as colicinogenic factors, can be established and maintained in the *lon*, as well as in the *lon*$^+$, strains (my unpublished results). This implies that establishment or maintenance of these plasmids involves processes different from those required by P1 and $\lambda gal N^-$ plasmids.

The finding with the *lon*$^+$ revertants show that the function required for plasmid formation is affected by the same pleiotropic mutation that causes the other *lon* effects: defective septum formation (6), high UV sensitivity (7), and overproduction of capsular polysaccharides (8). The *hcr*$^+$ and *rec*$^+$ functions are expressed normally in *lon* bacteria. Thus, the increased UV sensitivity of the *lon* strains is not due to a loss of capacity to reactivate UV-induced photoproducts in

TABLE 11. *CM^R transduction by P1CM and P1CMp*

Phage	CM^R transductants per ml on AX14 *lon*	CM^R transductants per ml on 2e10c *lon*$^+$
P1CM	2.3×10^3	5.0×10^7
P1CM*p*3a	1.7×10^7	7.5×10^7
P1CM*p* No. 4111	3.6×10^6	2.3×10^7

DNA (7). The UV sensitivity of the *lon* bacteria may be due to an abnormal sensitivity of some step in the process of septum formation.

Several hypotheses about the nature of the *lon* mutants have been proposed (7, 8, 14, 15, 21, 22). None of them provides a direct interpretation of the defect of plasmid formation in these mutants. The following suggestions may be entertained.

The bacterial membrane may play a critical role in the persistence or the establishment of plasmids. The abnormal septum formation and overproduction of capsular polysaccharides in the *lon* mutants might reflect an alteration in some membrane property. Attachment of the plasmids to specific site of the membrane might be needed for the replication of the plasmids (23, 24). Therefore, alteration of some membrane property in the *lon* mutants might cause the defect in plasmid formation. Hirota, Ryter, and Jacob (24) found bacterial mutations that prevented the autonomous replication of the sex factor and produced alterations on the bacterial membrane. There is much evidence relating the vegetative replication of various phages to the bacterial membrane (25–27).

Some regulatory mechanism involved in the "decision" to establish the plasmid state might be altered in the lon bacteria. Since λ^+ and P1 can grow vegetatively on the *lon* bacteria, all the functions needed for vegetative growth of these phages must be expressed normally in the *lon* bacteria. In order to establish the persistent relation between the host bacterium and plasmid, some step of the vegetative development of these phages must be blocked. In the case of λ, the block is provided by a defect in the *N* cistron (2, 3); in P1 the blocking is due to the immunity repressor. One must assume that in the *lon* bacteria the blocking process fails to operate effectively because of loss of the lon^+ function. In fact, in the case of P1, practically all the infected *lon* bacteria enter the vegetative growth cycle, so that the immunity repressor either is not made or remains ineffective. In the case of λ, either the defect of the N gene may be bypassed (28, 29) in the absence of the lon^+ function, or, more likely, the establishment of the plasmid state may require the active function of lon^+.

A common function controls both the initiation of septum formation and the replication of plasmids. The coincidence of the defects in plasmid formation and septum formation by the *lon* mutation may suggest that initiation of septum formation and regulation of plasmid formation are controlled for a common factor produced by the lon^+ gene. Initiation of cellular division must be preceded by *de novo* protein synthesis after the completion of one cycle of chromosome replication (30). A factor that triggers the initiation of chromosomal replication and(or) septum formation might act also as a trigger for a new round of plasmid replication. An interesting possibility is that gene *min*A (17), which is located very close to *lon* and has an effect phenotypically opposite to that of *lon*, might be part of a cluster of regulator genes for cellular division.

The evidence at hand does not support or exclude any of these models. A genetic and physiological analysis of the phage mutants that can lysogenize on the *lon* strains is in progress.

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