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The Mutation that Makes *Escherichia coli* Resistant to λ *P* Gene-mediated Host Lethality Is Located within the DNA Initiator Gene *dnaA* of the Bacterium

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Earlier, we reported that the bacteriophage λ *P* gene product is lethal to *Escherichia coli*, and the *E. coli rpl* mutants are resistant to this λ *P* gene-mediated lethality. In this paper, we show that under the λ *P* gene-mediated lethal condition, the host DNA synthesis is inhibited at the initiation step. The *rpl8* mutation maps around the 83 min position in the *E. coli* chromosome and is 94% linked with the *dnaA* gene. The *rpl8* mutant gene has been cloned in a plasmid. This plasmid clone can protect the wild-type *E. coli* from λ *P* gene-mediated killing and complements *E. coli dnaAts46* at 42°C. Also, starting with the wild-type *dnaA* gene in a plasmid, the *rpl*-like mutations have been isolated by *in vitro* mutagenesis. DNA sequencing data show that each of the *rpl8*, *rpl12* and *rpl14* mutations has changed a single base in the *dnaA* gene, which translates into the amino acid changes N313T, Y200N, and S246T respectively within the DnaA protein. These results have led us to conclude that the *rpl* mutations, which make *E. coli* resistant to λ *P* gene-mediated host lethality, are located within the DNA initiator gene *dnaA* of the host.

Keywords: DNA replication, *dnaA* gene of *E. coli*, Lambda *P* gene, λ *P* lethality, *rpl* mutation.

Introduction

Bacteriophages have evolved the genetic systems, which may specifically interact with certain host gene products to exploit the latter for their own growth. These types of genetic and physiological interactions of bacteriophage λ with its host *Escherichia coli* have been extensively studied (Friedman *et al*., 1984). Thus, (a) the N and Q proteins of λ interact with the Nus protein(s) and RNA polymerase of *E. coli* to antiterminate phage-specific transcriptions from two early promoters *p*L and *p*R and the late promoter *p*R' respectively (Friedman and Court, 1995); (b) the λ-coded CIII protein inhibits the Hfl protein of host to stabilize the λ CII protein which is essential for the establishment of lysogeny (Echols, 1986); (c) the interactions of λ P protein with DnaB, DnaJ and DnaK proteins of the host are essential for λ DNA replication (Dodson *et al*., 1989; Libereck *et al*., 1990); (d) the assembly of phage head requires the assistance of GroEL and GroES proteins of *E. coli* (Georgopoulos and Hahn, 1978); and (e) the activated RecA protein of *E. coli* cleaves the λ repressor to effect prophage induction during an SOS response (Roberts *et al*., 1978). It appears that all of the above interactions are beneficial for λ growth; only CII protein has been reported to be toxic to the host (Kedzierska *et al*., 2001).

Lambda does not show productive growth in the absence of its *N* gene function (Signer, 1969). Infection of a nonpermissive *E. coli* by $\lambda N^{-}c\mathrm{I}^{-}$ phage does not induce host killing even at a super-high multiplicity of infection (MOI) of 100 or more (Chattopadhyay *et al*., 1983), while induction of a $\lambda N^{-}c\mathrm{I}ts$ lysogen at 42°C leads to bacterial killing that requires the phage replication genes *O* and *P* as well as the right promoter *p*R (Sly *et al*., 1968). However, the probability of the latter type of bacterial killing by λ decreases with an increase in the number of integrated $\lambda N^{-}c\mathrm{I}ts$ prophages per host chromosome in polylysogens (Lieb, 1972). A lethal gene called *kil* located between γ and *c*III genes in the left operon of λ has been reported (Greer, 1975b), and the lethal action of the *kil* gene is dependent on the *N* gene function but not on phage DNA replication (Greer, 1975a). The *kil* gene product possibly interacts with certain component (s) of the envelope (Greer, 1975b) and inhibits cell division (Sergueev *et al*., 2001).

It was reported from our laboratory that the DNA

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replication gene *P* of λ causes bacterial killing even in the absence of phage DNA replication (Maiti *et al*., 1991a). To understand the mechanism of λ *P* gene-induced host killing, *E. coli rpl* mutants resistant to λ *P* gene lethality were isolated (Maiti *et al*., 1991b). Of the four such mutants, *rpl1* and *rpl7* were also resistant to λ superinfection, and were later found to be λ lysogens, and hence, these were rejected. The two λ sensitive *rpl* mutants, *rpl8* and *rpl9*, were identical in their different properties (Maiti *et al*., 1991b), and only one of them, *E. coli* 594*rpl8* was used to identify the host gene that is involved in λ *P* gene-mediated lethality. In this paper, we present evidences, which lead us to conclude that the *rpl* mutations are located in the DNA initiator gene *dnaA* of *E. coli.* This suggests that the DnaA protein of the host is the target of λ *P* gene-mediated lethality.

Materials and Methods

Media, bacterial and bacteriophage strains and growth conditions and DNA isolation The compositions of tryptone broth (TB), tryptone broth with maltose (TBM), phage dilution medium, tryptone agar and soft agar are described in Chattopadhyay and Mandal (1982), and those of Luria-Bertani (LB) broth and Luria agar (LA) are as described (Sambrook *et al*., 1989).

E. coli K-12 strains, 594 ($galK^-$ *galT* lac^- thi^- Str^R su^-) was obtained from M. Lieb (1972), DH5 ($endI^-r^-m^+$ $supII^+$ thi^- *recA* gyr^- *rel)*, and *dnaAts46 (metE46 trp8 thi-1 galK2 lacY1 mtl-1 ara-9 tsx-3 rpsL8 supE44)* from S. Adhya, and BR1639 (*recA56::Tn10* $lacI^q$ *lacZ::Tn5),* Q18 (*dnaBts thyA lacY supE44 thr leu thi met*) and BR2965 (*leuB6 thyA47 draT12 rpsL153 dnaCts deoC3*) from D. Chattoraj. *E. coli 594rpl8* is an *rpl* mutant derivative of 594 (Maiti *et al*., 1991b). The 594 (λ112) and 594*rpl8* (λ112) lysogens were isolated during this work. The λN^-cI^-*hk* (λ*Nam7am53c*I60*hk)* phage was used from laboratory stock [in the presence of *hk* mutation in the *cro* gene, even in the absebce of *N* gene function, this phage expresses the P protein to an elevated level that causes bacterial killing (Maiti *et al.,*1991a)]. The λ112 (λ*imm*21*c*I^+*p*$RM^λ$*c*$I857^λ$*lacZ* fusion) phage was obtained from M. Ptashne (Maurer *et al.,* 1980) and P1 (P1*clr*100cm^R) from S. Adhya.

E. coli cultures were routinely grown in TB, TBM (for λ infection experiments) or LB at 37°C with shaking, and the growth was monitored by measuring the OD of the culture at 590 nm. The bacteria harboring plasmid were grown in the presence of required antibiotic [chloramphenicol (Cm) at 35 µg/ml or ampicillin (Amp), at 50 µg/ml]. The lysogens of λ and λ112 were prepared by isolating the bacteria from the turbid center of the plaques formed by the phage on the bacteria. These were purified by single colony method and confirmed by their immunity to the respective homoimmune phages but not to the heteroimmune λ*i*434 phage. Bacteriophage λ was prepared by growth and lysis in permissive *E. coli* in TBM, concentrated by PEG precipitation and purified by CsCl banding, and the phage DNA was isolated following the methods as described (Sambrook *et al*., 1989). *E. coli* DNA was prepared by the method of Marmur (1961) and plasmid DNA, by the procedure as described by Sambrook *et al.* (1989).

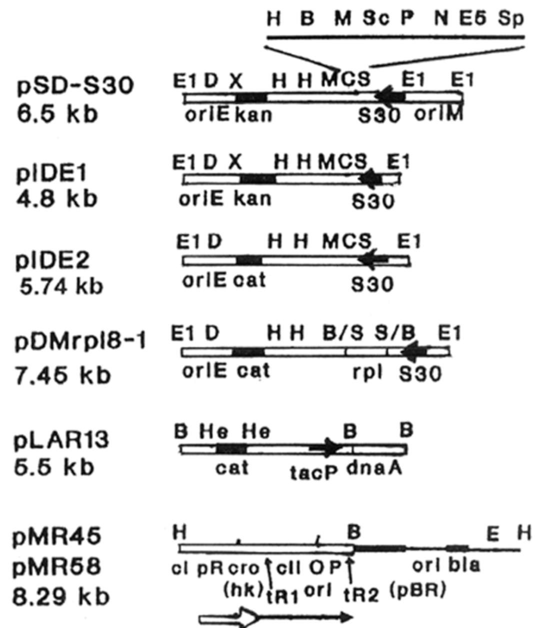

Fig. 1. Maps of different plasmids**.** Maps of the plasmids are shown in linearized forms nearly to the same scale. Sizes of the plasmids in kb are indicated by the numbers written below the names of the plasmids at the left. Approximate positions of the restriction enzyme cleavage sites are indicated by the enzyme symbols written above the map and those of different important genes and replication origins by the gene and *ori* symbols written below the map. The arrows show the directions of promoters. The restriction enzyme cleavage sites within the multiple cloning sites (MCS) in pSD5-S30 plasmid are shown in the expanded region above the map. *oriE* indicates p15A origin compatible for replication in *E. coli* and *oriM* for replication in mycobacteria. The restriction enzyme symbols: B, *Bam*HI; D, *Dra*I; E1, *Eco*RI; E5, *Eco*RV; H, *Hin*dIII; He, *Hae*II; M, *Mlu*I; N, *Nde*I; P, *Pst*I, S, *Sau*3AI; Sc, *Sca*I; Sp, *Sph*I; and X, *Xho*I.

Plasmids The structures of different plasmids are shown in Fig. 1. Recombinant DNA methods used were as described (Sambrook *et al*., 1989). The plasmid pMR45 (Fig. 1; Maiti *et al*., 1991a) contains the *Hin*dIII/*Bam*HI 4.27 kb segment of λN^-cI^-*hk* phage DNA spanning from the middle of *c*I to the right side of the *P* gene cloned in pBR322 DNA. Due to the presence of *hk* mutation in the *cro* gene, the expression of *P* gene from the *p*R promoter occurs to a lethal level from the above λ DNA segment in pMR45 in a nonlysogenic *E. coli* but not in a λ lysogen. The plasmid pMR58 is a P^- derivative of pMR45 (Maiti *et al*., 1991a). The plasmid pLAR13 (Rokeach and Zyskind, 1986) contains the wild-type *dnaA* gene flanked by two *Bam*HI sites and tagged to the *tac* promoter (Fig. 1). The medium copy number plasmid pIDE1 was constructed by self ligating the *Eco*RI-generated 4.8 kb fragment containing kan^R and *ori*E (15A origin) of pSD5-S30 DNA (Fig. 1, Das Gupta *et al.,* 1993) and selecting in *E. coli* DH5. The plasmid pIDE2 was made by replacing the *kan* gene in pIDE1 by the *cat* gene from pLAR13 plasmid. The 4.48 kb fragment from *Hin*dIII/*Xho*I-digested pIDE1 DNA was gel purified and end filled by Klenow DNA Pol I reaction. The 1.26 kb DNA fragment containing the *cat*

gene was gel purified from *Hae*II-digested pLAR13 DNA and ligated with the above end-filled 4.48 kb DNA. This was then transformed into DH5, and the plasmid pIDE2 (5.74 kb) was selected on Cm plate.

Transformation, complementation, and λ *P* gene sensitivity of *E. coli* Transformation of *E. coli* with plasmid DNA (around 100 ng) was done either by using $CaCl_2$-competent cells (Sambrook *et al*., 1989) or by electroporation (Gene Pulsar, BIO-RAD). *E. coli* 594 or its derivative, when used as recipient for transformation, was grown to 0.9-1.0 OD_{590} and then made competent. To check the complementation ability of a plasmid carrying a heat-stable *dnaA* gene, the *E. coli dnaAts46* mutant was transformed with the plasmid, and the transformants were plated on suitable drug plate at 42ºC. The complementation was considered as positive when the transformation efficiency of the experimental plasmid was nearly equal to that of the control plasmid pLAR13 DNA (*dnaA*$^+$). Bacteriophage λ *P* gene sensitivity of *E. coli* was tested by comparing the survival of the experimental bacteria following transformation with pMR45 DNA (Maiti *et al*., 1991a) with that of the control *rpl8* bacteria under identical conditions of λ P protein (λ P protein will be called simply P in the text) challenge by transformation with pMR45 DNA. The λ *P* gene sensitivity was also tested by determining the survival of the bacteria following infection with λN^-cI$^-$*hk* phage at around 50 multiplicity (Maiti *et al*., 1991a).

Transduction The bacteriophage P1 was grown on *E. coli* 594*rpl8* (donor). Transduction of the *rpl* mutation from the above donor bacterium to different recipients was done using the above P1 phage stock by the procedure as described by Silhavy *et al*. (1984). The recipient *E. coli* strains carried temperature-sensitive mutations in the marker genes, and the wild-type transductants were selected at 42ºC. Cotransduction frequencies of the unselected *rpl* marker with different selected markers were determined by comparing the survival of the selected transductants with that of the *rpl* bacteria following challenge with pMR45 DNA under identical conditions.

Cloning of the *rpl8* mutant gene The gene carrying the *rpl8* mutation was cloned as follows: The *E. coli* 594*rpl8* chromosomal DNA was partially digested with *Sau*3AI, and the DNA fragments in the size range of 1.5-4.0 kb were purified from agarose gel. The *Bam*HI-digested pIDE2 DNA was treated with calf intestinal phosphatase and ligated with the above 1.5-4.0 kb DNA fragments. The ligated DNA was electroporated into *E. coli* DH5, and the transformants were selected on Cm plate. About 200,000 colonies were pooled and grown to 1 OD_{590} in TB containing Cm, and the plasmid DNAs were isolated. This gave the *Sau*3AI library of *E. coli* 594*rpl8* genomic DNA in pIDE2 plasmid.

The wild-type *E. coli* (*rpl*$^+$) is sensitive to killing by the λ *P* gene product (Maiti *et al*., 1991a), while the *rpl8* mutant is not (Maiti *et al.* 1991b). This suggests that the *rpl8* mutation is dominant over its wild-type (*rpl*$^+$) allele. So, the wild-type *E. coli* (*rpl*$^+$) harboring the plasmid that contains and expresses the *rpl8* mutant gene would survive the challenge by λ *P* gene product. This defined the strategy of selection of the plasmid clone containing the *rpl8* mutant gene. Using this strategy, the *rpl8* genomic library was screened to get the putative *rpl* mutant gene clone as follows: A small amount of plasmid from the above library was electroporated into *E. coli* 594 and plated on LA containing Cm. About 200,000 transformant colonies were pooled and grown in LB containing the drug. An aliquot of this culture was challenged with pMR45 (Amp^R) and plated on Cm + Amp agar. As the *rpl8* mutant shows a slow growing phenotype (S. Banik-Maiti and N. C. Mandal, unpublished results), around 30 tiny colonies were selected from among those surviving on Cm + Amp plate (survival was around 0.35% of that of the *E. coli* 594*rpl8* bacteria under identical conditions of pMR45 challenge). Plasmids were isolated from those individual colonies. To eliminate pMR45 (Amp^R), a small amount of each of the above isolated plasmid was electroporated into *E. coli* 594 and plated on LA containing only Cm. The colonies which were resistant to Cm but not to Amp were purified from each set and challenged with λ *P* gene product by transformation with pMR45. By this procedure, two plasmid clones were identified, which protected the harboring bacteria from killing by P. These were called pDMrpl8-1 (Fig. 1) and pDMrpl8-2.

***In vitro* mutagenesis of the *dnaA* gene** The pLAR13 DNA containing the wild-type *dnaA* gene was treated with 100 µg/ml of hydroxyl amine (Silhavy *et al*., 1984) to reduce the transformation efficiency of the plasmid to around 5% of that of the untreated parent plasmid and electroporated into *E. coli* BR1639. About 200,000 transformant colonies were pooled from LA + Cm plate, and the plasmid DNA was isolated. To eliminate the mutation(s) in the upstream promoter or anywhere in the plasmid other than those in the *dnaA* gene, this pooled plasmid DNA was digested with *Bam*HI, and the 1.5 kb DNA fragment containing the *dnaA* gene without its upstream promoter (Fig. 1) was gel purified and ligated with the gel-purified 5.65 kb DNA fragment from the *Bam*HI-digested pIDE2 DNA. The ligated DNA was electroporated into *E. coli* DH5, and the total transformants (about 10,000 colonies) formed on Cm plates were pooled. These were grown in LB containing Cm, transformed with pMR45 and plated on Cm + Amp agar. The survival of the transforming bacteria was around 11% (relative to the *E. coli* 594*rpl8* mutant). From among these transformants, 30 tiny colonies were selected, and the plasmid DNAs were isolated from each of them. To eliminate pMR45 plasmid (Amp^R), a small amount of each of these 30 plasmid isolates was electroporated into *E. coli* 594 (wild type) and plated on LA containing only Cm. The colonies resistant to Cm but not to Amp were purified from each set. These were individually tested for the *rpl* property by transformation with pMR45. Several of these bacteria harboring the plasmid showed resistance to P. Three of them were used in further studies. These were called pDMrpl10, pDMrpl12, and pDMrpl14. The structures of these plasmids are the same as that of pDMrpl8-1 (Fig. 1) except that the 1.9 kb DNA insert in the latter is replaced by 1.5 kb DNA in the former three.

Southern blot and DNA sequencing The 1.3 kb DNA segment flanked by *Eco*RI and *Eco*RV within the wild-type *dnaA* gene in pLAR13 DNA (Fig. 1) was gel purified and labeled with [^{32}P] using [α-^{32}P]dATP (obtained from BRIT, Trombay, Mumbai) by the random primer labeling method (Sambrook *et al*., 1989). The DNA to be blotted was digested with *Eco*RI and *Eco*RV, and the DNA fragments were separated by electrophoresis on a 0.8% agarose gel and blotted with the above-labeled DNA probe by the procedure as

described (Sambrook *et al*., 1989). DNA sequencing was done using pDMrpl8-1, pDMrpl12, and pDMrpl14 DNAs as templates. Four different 20 mer primers were designed from the known sequence of the *dnaA* gene (Hansen *et al*., 1982) in such a way that the whole of the *dnaA* gene containing DNA segment was sequenced. Sequencing was done in an automatic DNA sequencer (Applied Biosystem, Foster City, USA).

Radioactivity measurements Radioactive counts were determined in a Beckman LS 5000CE counter (Fullerton, USA).

Results

DNA synthesis in wild-type *E. coli* is inhibited under the condition λ *P* gene-mediated lethality Since the P protein of λ is essential for its own DNA replication during which it physically interacts with the DnaB protein (the DnaB protein will be called as DnaB only in the text) of *E. coli* (Dodson *et al*., 1989; Libereck *et al*., 1990), we examined the DNA synthesis in wild-type *E. coli* under the condition of λ *P* gene-mediated lethality. The plasmid pMR45 expresses the λ *P* gene from the *p*R promoter creating a lethal environment in nonlysogenic *E. coli* (Maiti *et al*., 1991a), but in 594 (λ112) lysogen growing at 32ºC, the transcription of the *P* gene from *p*R in pMR45 is completely inhibited by the CI*ts*857^{λ} repressor supplied in *trans* from the λ112 prophage (λ112 prophage is maintained by λ*imm*21 phage-specific repressor and is not inducible at 42ºC; Maurer *et al*., 1980). But at 42ºC, the above plasmid causes bacterial killing in 594 (λ112) because the CI*ts*857 repressor is inactivated at this temperature (killing data not shown). The data in Fig. 2 (A) show that at 42ºC, the DNA synthesis in 594(λ112) was inhibited in the presence of pMR45 (P^+) but not in its absence. In the same lysogen harboring pMR58 (P^-) or in 594*rpl8* (λ112) harboring pMR45 (P^+), the DNA synthesis was not inhibited at 42ºC. We conclude that λ *P* gene product inhibits bacterial DNA synthesis in wild-type *E. coli* but not in its *rpl* mutant derivative.

The results in Fig. 2(B) show that in a synchronized culture of *E. coli* 594 (λ112) lysogen harboring pMR45(P^+), the DNA synthesis was inhibited only when P was provided (by heat

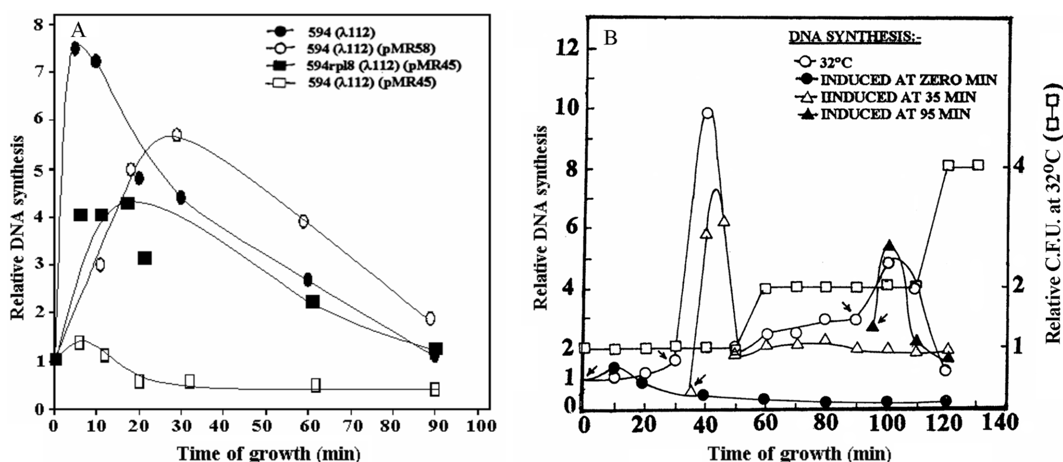

Fig. 2. Effect of the lethal level of the λ *P* gene product on *E. coli* DNA synthesis in nonsynchronized (A) and synchronized (B) cultures**.** (A), bacteria were freshly grown to around 0.2 OD_{590} at 32ºC with shaking and then two 5 ml aliquots were taken out. One was incubated at 32ºC and the other at 42ºC. At desired times, 0.05 ml aliquots from both the sets were taken out in separate tubes and pulsed with 0.5 µCi [^{3}H]-thymidine (17.2 Ci/mmol, obtained from BRIT, Trombay, Mumbai) at 32 or 42ºC for 3 min, and then 1 ml of 10% TCA was added and chilled on ice. The TCA-insoluble radioactivity was determined. Only the results of the experiments done at 42ºC have been presented. All of the corresponding 32ºC sets showed DNA synthesis (data not shown). At zero time, the [^{3}H] incorporations into DNA were around 2,500-3,500 cpm/mL/3 min for different cultures. In each set, the DNA synthesis data relative to their respective zero time values are presented. (B), The bacteria [*E. coli* 594(λ112) harboring pMR45] were grown to around 0.2 OD_{590} at 32ºC and then synchronized by growing further for 2 h in the presence of 15 µg/ml of Cm (at 32ºC). Then the culture was chilled, and the cells were washed twice (to eliminate the drug) and suspended in a drug-free medium (TB) to around 0.15 OD_{590}. This cell suspension was incubated at 32ºC with shaking. At indicated times, 5 ml aliquots were taken out and induced at 42ºC for 15 min and then incubated at 39ºC. At different times, 0.05 ml aliquots were taken out from both 32ºC (control) and 39ºC (induced) sets and pulsed with 0.5 µCi of [^{3}H] thymidine (17.2 Ci/mmol) for 3 min at the respective temperatures of their immediate previous growth. The TCA-insoluble radioactivities were then determined. At different times, viable cell numbers were determined from the set continuously growing at 32ºC. In the DNA synthesis data curves, the rightward arrows (inclined downwards) at 30 and 90 min indicate the points of initiation of the rounds of DNA replication (which precedes cell division), and the leftward arrows (inclined downwards) at zero, 35, and 95 min indicate the points at which inductions were made. At zero time, the viable cell count was around 10^7 CFU/ml and TdR incorporations into DNA were around 3,000-4,000 cpm/ml/3 min. In each set, the DNA synthesis data relative to their respective zero time values have been presented. For other details, see Methods.

Table 1. The map position of *rpl* mutation in the *E. coli* chromosome

Recipient *E. coli* strain	Selected marker: From *rpl* donor	Selected marker: Map position	Unselected *rpl* marker: Cotransduction frequency	Unselected *rpl* marker: Approximate map position
*dnaAts*46	*dnaA*$^+$	83 min	90/96 (94%)	83 min
dnaBts	*dnaB*$^+$	92 min	0/50	----
dnaCts	*dnaC*$^+$	99 min	0/50	----

For details, see Methods.

induction) before the initiation of a round of bacterial DNA replication. Induction made just after the initiation of a round of replication could not inhibit the synthesis (elongation) of DNA in that initiated round but did inhibit the initiation of the subsequent rounds. This suggests that under P-mediated lethal condition, the initiation (and not the elongation) of host DNA synthesis is inhibited.

The *rpl8* mutation is located within the *dnaA* gene of *E. coli* As the *P* gene product of λ at its lethal level inhibits host DNA synthesis (Fig. 2), the target gene of host for λ *P* gene-mediated lethality is, possibly, essential for the bacterial DNA synthesis. Since λ plates on the *rpl8* mutant bacteria (Maiti *et al.*, 1991b), it may be assumed that the above referred target gene of host would be the one among those which are essential for *E. coli* DNA replication but not for λ DNA synthesis. An examination of the list of the genes of *E. coli* required for the bacterial and/or λ DNA synthesis reveals that there are at least two genes, *dnaA* and *dnaC*, which are essential for the initiation of DNA synthesis from *oriC* region of *E. coli* but not for λ DNA synthesis (Furth and Wickner, 1983). So, we chose the *dnaAts* and *dnaCts* mutants of *E. coli* as the recipients in bacteriophage P1 transduction using *E.coli* 594*rpl8* as the donor. Also, the *E. coli dnaBts* strain was used as a recipient (because the wild-type DnaB physically interacts with P). In all the cases, the corresponding wild-type (*dnaA*$^+$, *dnaB*$^+$ or *dnaC*$^+$) alleles were selected at 42°C, and the co-transduction frequencies of the unselected *rpl* marker with the above selected markers were determined. The results in Table 1 show that the *rpl8* mutation is around 94% linked with the *dnaA* gene but not with the *dnaC* and *dnaB* genes. These results suggest that the *rpl* mutation maps around the 83 min position on the *E. coli* chromosome and is located very close to *dnaA* if not within this gene. However, the observation that P inhibits bacterial DNA synthesis at the initiation step [Fig. 2(B)], and that the *rpl8* mutation is closely linked to the DNA initiator gene *dnaA* of the bacteria, strongly suggest that the above mutation resides within the *dnaA* gene. This was confirmed by cloning the *rpl8* mutation as well as by isolating the *rpl*-like mutations in the *dnaA* gene by *in vitro* mutagenesis and characterizing them, as described below.

By screening an *rpl8* genomic library prepared in a medium copy number plasmid pIDE2, two putative clones, called pDMrpl8-1 and pDMrpl8-2, which showed the *rpl* phenotype

Table 2. Lambda P-resistance property of different plasmids

Bacteria[a]	Survival after transformation with pMR45 DNA[b]: CFU/mg of plasmid DNA	% of control
594 *rpl8*	9.03×10^5	100
594 (wild type)	<3.00×10^2	<0.03
594 (pLAR13)	<3.00×10^2	<0.03
594 (pIDE2)	<3.00×10^2	<0.03
594 (pDMrpl8-1	9.00×10^5	99.5
594 (pDMrpl8-2)	9.69×10^5	107
594 (pDMrpl10)	9.03×10^5	100
594 (pDMrpl12)	8.20×10^5	90.8
594 (pDMrpl14)	8.20×10^5	90.8

[a]All the plasmids had *cat* marker.
[b]The bacteria ($CaCl_2$-competent) under first column were transformed with about 100 ng pMR45 DNA (Amp^R). Transformed 594*rpl8* and 594 were plated on Amp plate and all others on Amp+Cm plates all at 37°C. The 594*rpl8* bacteria were used as the P-resistant control.

were obtained (see Methods). The survival of *E. coli* 594 (wild type) harboring each of these plasmids was nearly similar to that of the *rpl8* mutant when challenged with P by transformation with pMR45 under identical conditions (Table 2, lines 1-6). Both these plasmids also complemented *E. coli dnaAts46* mutant at 42°C (Table 3, lines 1-5). By restriction analysis, the sizes of the DNA inserts in pDMrpl8-1 and pDMrpl8-2 were determined to be 1.9 and 2.8 kb respectively, and the 1.9 kb DNA insert in pDMrpl8-1 and the DNA segment containing the wild-type *dnaA* gene in pLAR13 plasmid showed exactly identical cleavage-site maps with respect to *Dra*I, *Eco*RI, *Eco*RV, *Pvu*I, and *Pvu*II (data not shown). The insert DNA segments carrying the *rpl* mutation in the above two plasmids gave positive Southern blot signals with the *dnaA* gene-specific DNA probe from pLAR13 DNA (Fig. 3). All the above results led us to conclude that the *rpl* mutation is present within the *dnaA* gene.

The above conclusion was verified also by isolating the *rpl*-like mutations by *in vitro* mutagenesis of the *dnaA* gene in a plasmid (see Methods). Three plasmids, pDMrpl10, pDMrpl12 and pDMrpl14 were obtained, which protected the wild-type

Table 3 Complementation of *dnaAts46* mutation by different plasmids

Recipient bacterium	Transforming plasmid	Survival at 42ºC after transformation (% of control at 32ºC)
E. coli dnaAts46	None	<0.07
	pLAR13	97.1
	pIDE2	<0.07
	pDMrpl8-1	98.3
	pDMrpl8-2	97.1
	pDMrpl10	97.5
	pDMrpl12	98.5
	pDMrpl14	114

E. coli dnaAts46 was grown at 32ºC and transformed with different plasmids and grown, and plated in duplicates. One from each set was incubated at 32ºC and the other at 42ºC. The colony counts of the plasmid transformants for all the 32ºC sets were around 5.5×10^6 CFU/mg plasmid DNA. For other details, see Methods.

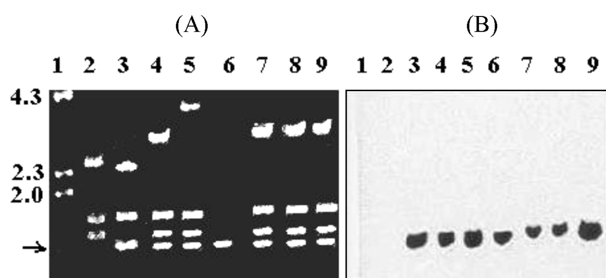

Fig. 3. Southern blot of the *rpl* clones digested with *Eco*RI and *Eco*RV and probed with [^{32}P]-labelled *Eco*RI-*Eco*RV 1.3 kb DNA from the wild-type *dnaA* gene sequence in pLAR13 plasmid. A, EtBr-stained DNA bands; B, autoradiogram of the blots. The positions of DNA bands in A showing blots in B are indicated by arrow. Lanes: 1, λ/*Hin*dIII; 2, pIDE2; 3, pLAR13; 4, pDMrpl8-1; 5, pDMrpl8-2; 6, purified 1.3 kb probe from *dnaA* gene sequence containing DNA; 7, pDM14; 8, pDM12; 9, pDM10. For other details, see Methods.

E. coli from λ *P* gene-induced killing (Table 2, lines 7-9) and complemented the *E. coli dnaAts46* mutant at 42ºC (Table 3, lines 6-8). These results suggest that the P-resistance property of *E. coli* is acquired by the *rpl*-like mutations in the *dnaA* gene and that these mutations are *trans*-dominant to both wild-type *dnaA* gene and its *ts46* mutant derivative.

Identification of the positions of *rpl* mutations by DNA sequencing To determine the locations of the *rpl* mutations in the *dnaA* gene, the *rpl8, rpl12* and *rpl14* DNA segments (in plasmids) were sequenced. The sequencing data in Fig. 4 indicate that in each of these *rpl* mutants, a change of single base occurs in the 313th *(rpl8)*, 200th *(rpl12)*, and 246th (*rpl14*) codons in the *dnaA* gene, which translate into the amino acid changes from Asn (N) to Thr (T), Tyr (Y) to Asn (N), and

rpl8 → **CODON No. 313 changed from AAC to ACC**
Amino acid changed from Asn to Thr

rpl12 → **CODON No. 200 changed from UAU to AAU**
Amino acid changed from Tyr to Asn

rpl14 → **CODON No. 246 changed from UCU to ACU**
Amino acid changed from Ser to Thr

Fig. 4. Changes of bases and amino acids due to *rpl* mutations Changes of bases in the particular codons by the *rpl* mutations and the corresponding amino acid changes are shown.

from Ser (S) to Thr (T) respectively in the DnaA protein (the DnaA protein will be called as DnaA in the text).

Discussion

In this paper, we show that the *rpl* mutations are located within the DNA initiator gene *dnaA* of *E. coli*. The fact that due to the presence of these mutations in the *dnaA* gene, the harboring bacterium becomes resistant to λ *P*-gene-induced killing suggests that the P protein of λ targets the host DNA initiator protein DnaA to show the lethal effect.

The two λ genes *O* and *P* are essential for DNA replication specifically from ori^{λ} (Furth and Wickner, 1983). The roles played by the O and P proteins in the above event are exactly similar to those played by the DnaA and DnaC proteins (the DnaC protein will be called as DnaC in the text) respectively of *E. coli* in the initiation of DNA replication from the homologous origin *oriC*. The DnaB protein of *E. coli* physically interacts with each of P (Libereck *et al*., 1990) and DnaA (Marszalek and Kaguni 1994). The O protein binds to the ori^{λ} site, and P brings and loads DnaB onto the ori^{λ}-bound O protein to form the preprimosome (Dodson *et al*., 1989; Libereck *et al*., 1990). In an analogous way, DnaA binds to several 9-mer DnaA boxes within the *oriC* region of *E. coli* DNA, and the DnaC protein brings and loads DnaB onto the *oriC* DNA-bound DnaA to form the preprimosome (Fang *et al*., 1999). So, there was a possibility that DnaC and P may compete for their interactions with DnaB and that the P protein at its lethal level either inactivates DnaC or simply competes it out from interaction with DnaB, thereby inhibiting the bacterial DNA synthesis, which leads to bacterial death. Surprisingly, however, it turned out that the λ *P* gene targets the *dnaA* gene for its lethal effect on the host cell. The P protein has higher affinity for DnaB than that of DnaC for DnaB (Mallory *et al*., 1990). Whether this competition between P and DnaC for DnaB causes any lethal effect is not known. It may be that P interacts with both DnaA and DnaB to inhibit host DNA synthesis, while the interaction of P with only DnaB directs the bacterial DNA synthesis machinery in favor of phage DNA synthesis. In *E. coli groP* mutant, the λ P protein does not interact with DnaB, yet this bacterium is sensitive to P-mediated lethality (Maiti *et al*.,

1991a). This also rules out the possibility that the interaction between P and DnaB is lethal to host. The three *rpl* mutations have been located in the segment from 200th to 313th codons of the DnaA protein. This region of DnaA happens to overlap with the RepA protein-interaction domain (Sutton and Kaguni, 1995; see Datta *et al.*, 2004). This suggests that both the λ-coded P protein and the plasmid pSC101-coded RepA protein target the same region (domain) of DnaA for their interactions.

Damaging interaction of certain phage gene products on the composite structure of the host cell organelle may be lethal. Thus the disrupting action of phage lysis genes on host cell membrane and wall is always lethal. The bacteriophage T4 inhibits the host DNA replication by disrupting the nucleoid organization of the host by the phage-coded *ndd* gene product; this causes the shutdown of host RNA and protein syntheses by inducing premature termination of transcription of certain essential genes and degrading mRNA respectively (Kutter *et al*. 1994). This type of T4-induced host inhibition or host shutoff is lethal to its host. In the *B. subtilis* phage SPO1, the phage-coded *e3* gene product inactivates the β subunit of host RNA polymerase and causes host killing (Wei and Stewart, 1993; 1995). Also, the Cp10 phage-coded RNA polymerase-binding protein p7 inhibits the transcription initiation and termination by host *Xanthomonas oryzae* RNA polymerase (Nechaev *et al*., 2002). Whether this inhibitory interaction of p7 protein is lethal to host is not yet known. As the *dnaA* gene plays a very crucial role in the initiation of DNA replication from *oriC* DNA, the lethal action of P by an interaction with DnaA of *E. coli* appears to be novel and unique. Under the P-mediated lethal condition, the initiation (and not the elongation) of bacterial DNA replication is inhibited (Fig. 2). In the accompanying paper (Datta *et al*., 2005), we have shown that the P protein of λ specifically inhibits the binding of DnaA to *oriC* DNA and ATP. So, the bacterial killing effect of the P protein of λ is possibly caused by the inhibition of initiaion of DNA replication by inhibiting the binding of DnaA to *oriC* DNA and ATP (see Datta *et al*., 2005 for further discussion).

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