The Mutation that Makes Escherichia coli Resistant to \( \lambda P \) 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 \( \lambda P \) gene product is lethal to Escherichia coli, and the E. coli rpl mutants are resistant to this \( \lambda P \) gene-mediated lethality. In this paper, we show that under the \( \lambda P \) 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 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 \( \lambda P \) 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, \( \lambda P \) 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 \( \lambda \) with its host

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Escherichia coli have been extensively studied (Friedman et al., 1984). Thus, (a) the N and Q proteins of \( \lambda \) interact with the Nus protein(s) and RNA polymerase of E. coli to antiterminate phage-specific transcriptions from two early promoters \( pL \) and \( pR \) and the late promoter \( pR' \) respectively (Friedman and Court, 1995); (b) the \( \lambda \)-coded CIII protein inhibits the Hfl protein of host to stabilize the \( \lambda \) CII protein which is essential for the establishment of lysogeny (Echols, 1986); (c) the interactions of \( \lambda P \) protein with DnaB, DnaD and DnaK proteins of the host are essential for \( \lambda \) 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 \( \lambda \) repressor to effect prophage induction during an SOS response (Roberts et al., 1978). It appears that all of the above interactions are beneficial for \( \lambda \) 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 \( \lambda N^cI^c \) 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 \( \lambda N^cI^cTS \) lysogen at 42°C leads to bacterial killing that requires the phage replication genes \( O \) and \( P \) as well as the right promoter \( pR \) (Sly et al., 1968). However, the probability of the latter type of bacterial killing by \( \lambda \) decreases with an increase in the number of integrated \( \lambda N^cI^cTS \) prophages per host chromosome in polylsogens (Lieb, 1972). A lethal gene called \( kil \) located between \( cI^c \) and \( cIII \) genes in the left operon of \( \lambda \) 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 (Sergeev et al., 2001).

It was reported from our laboratory that the DNA
replication gene $P$ of $\lambda$ causes bacterial killing even in the absence of phage DNA replication (Maiti et al., 1991a). To understand the mechanism of $\lambda$, $P$ gene-induced host killing, $E. coli$ rpl mutants resistant to $\lambda P$ gene lethality were isolated (Maiti et al., 1991b). Of the four such mutants, rpl1 and rpl7 were also resistant to $\lambda$ superinfection, and were later found to be $\lambda$ lysogens, and hence, these were rejected. The two $\lambda$ sensitive rpl mutants, rpl8 and rpl9, were identical in their different properties (Maiti et al., 1991b), and only one of them, $E. coli$ 594rpl8 was used to identify the host gene that is involved in $\lambda P$ 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 $\lambda P$ 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: galI lac thi StrR su+) was obtained from M. Lieb (1972), DH5 ($opi\tau^r$ m" sulI thi recA gyr-rep), and dnaA646 (netE46 trp8 thi-1 galK2 lacY1 metl-1 ara-9 tss-3 rpsL8 supE44) from S. Adhya, and BR1639 (recA56::Tn10 lacF lacZ::Tn5), Q18 (dnaBts thyA lacY supE44 thr leu thi met) and BR2965 (leuB6 thyA47 draf12 rpsL153 ducCts deoC3) from D. Chattoraj. $E. coli$ 594rpl8 is an rpl mutant derivative of 594 (Maiti et al., 1991b). The 594 (λ112) and 594rpl8 (λ112) lysogens were isolated during this work. The $\lambda N c1$ hK (λNam7am53cI60hK) phage was used from laboratory stock [in the presence of hK mutation in the cro gene, even in the absence of N gene function, this phage expresses the P protein to an elevated level that causes bacterial killing (Maiti et al., 1991a)]. The λ112 (λimm21c1\textsuperscript{F}pRM4857\textsuperscript{3}lacZ fusion) phage was obtained from M. Pashne (Maier et al., 1980) and P1 (P1cl100kmon) 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 homologous phages but not to the heterologous λ434 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).

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 HindIII/BamHI 4.27 kb segment of $\lambda N$ c1 hK phage DNA spanning from the middle of cI 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 PR 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 BamHI sites and tagged to the tac promoter (Fig. 1). The medium copy number plasmid pDE1 was constructed by self ligating the EcoRI-generated 4.8 kb fragment containing kan\textsuperscript{R} and oriE (15A origin) of pSD5-S30 DNA (Fig. 1, Das Gupta et al., 1993) and selecting in E. coli DH5. The plasmid pDE2 was made by replacing the kan gene in pDE1 by the cat gene from pLAR13 plasmid. The 4.48 kb fragment from HindIII/XhoI-digested pDE1 DNA was gel purified and end filled by Klenow DNA Pol I reaction. The 1.26 kb DNA fragment containing the cat

Fig. 1. Maps of different plasmids. The 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 pL5A origin compatible for replication in E. coli and oriM for replication in mycobacteria. The restriction enzyme symbols: B, BamHI; D, DraI; E1, EcoRI; E5, EcoRV; H, HindIII; He, HaelI; M, MluI; N, NdeI; P, PirI, S, Sau3AI; Sc, SceI; Sp, SphiI; and X, XhoI.
gene was gel purified from HaeII-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 CaCl₂-competent cells (Sambrook et al., 1989) or by electroporation (Gene Pulser, BIO-RAD). E. coli 594 or its derivative, when used as recipient for transformation, was grown to 0.9-1.0 OD₅₉₀ and then made competent. To check the complementation ability of a plasmid carrying a heat-stable dnaA gene, the E. coli dnaA₄₆ mutant was transformed with the plasmid, and the transfectants were plated on suitable drug plate at 42°C. The complementation was considered as positive when the survival of the selected transductants with that of the wild-type transductants was determined by comparing the complementation ability of a plasmid carrying a heat-stable dnaA gene.

**Transduction**  
The bacteriophage P₁ was grown on E. coli 594rpl8 (donor). Transduction of the rpl mutation from the 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 42°C. 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 bacterium 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 594rpl8 chromosomal DNA was partially digested with Sau3AI, and the DNA fragments in the size range of 1.5-4.0 kb were purified from agarose gel. The BamHI-digested pDIE2 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 OD₅₉₀ in TB containing Cm, and the plasmid DNAs were isolated. This gave the Sau3AI library of E. coli 594rpl8 genomic DNA in pDIE2 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 (Amp₃⁻) 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 594rpl8 bacteria under identical conditions of pMR45 challenge). Plasmids were isolated from those individual colonies. To eliminate pMR45 (Amp₃⁻), 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 hydroxylamine (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 BamHI, 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 BamHI-digested pDIE2 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 594rpl8 mutant). From among these transformants, 30 tiny colonies were selected, and the plasmid DNAs were isolated from each of them. To eliminate pMR45 plasmid (Amp₃⁻), 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 EcoRI and EcoRV within the wild-type dnaA gene in pLAR13 DNA (Fig. 1) was gel purified and labeled with [³²P] using [α-³²P]dATP (obtained from BRIT, Trombay, Mumbai) by the random primer labeling method (Sambrook et al., 1989). The DNA to be blotted was digested with EcoRI and EcoRV, 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 \( \lambda P \) gene-mediated lethality  Since the P protein of \( \lambda \) 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 \( \lambda P \) gene-mediated lethality. The plasmid pMR45 expresses the \( \lambda P \) gene from the \( pR \) promoter creating a lethal environment in non-lysogenic *E. coli* (Maiti et al., 1991a), but in 594 (\( \lambda 112 \)) lysogen growing at 32°C, the transcription of the \( P \) gene from \( pR \) in pMR45 is completely inhibited by the Clts857\(^{r} \) repressor supplied in *trans* from the \( \lambda 112 \) prophage (\( \lambda 112 \) prophage is maintained by \( \lambda \)imm21 phage-specific repressor and is not inducible at 42°C; Maurer et al., 1980). But at 42°C, the above plasmid causes bacterial killing in 594 (\( \lambda 112 \)) because the Clts857 repressor is inactivated at this temperature (killing data not shown). The data in Fig. 2 (A) show that at 42°C, the DNA synthesis in 594(\( \lambda 112 \)) was inhibited in the presence of pMR45 (\( P^{+} \)) but not in its absence. In the same lysogen harboring pMR58 (\( P^{+} \)) or in 594rpl8 (\( \lambda 112 \)) harboring pMR45 (\( P^{+} \)), the DNA synthesis was not inhibited at 42°C. We conclude that \( \lambda P \) 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 594 (\( \lambda 112 \)) lysogen harboring pMR45(\( P^{+} \)), the DNA synthesis was inhibited only when \( P \) was provided (by heat

Figure 2. Effect of the lethal level of the \( \lambda P \) gene product on *E. coli* DNA synthesis in nonsynchronized (A) and synchronized (B) cultures, (A), bacteria were freshly grown to around 0.2 OD\(_{660}\) at 32°C with shaking and then two 5 ml aliquots were taken out. One was incubated at 32°C and the other at 42°C. At desired times, 0.05 ml aliquots from both the sets were taken out in separate tubes and pulsed with 0.5 \( \mu Ci \) [\(^{3}H\)]-thymidine (17.2 Ci/mmol, obtained from BRIT, Trombay, Mumbai) at 32 or 42°C 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 42°C have been presented. All of the corresponding 32°C 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(\( \lambda 112 \)) harboring pMR45) were grown to around 0.2 OD\(_{660}\) at 32°C and then synchronized by growing further for 2 h in the presence of 15 \( \mu g/mL \) of Cm (at 32°C). 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\(_{660}\). This cell suspension was incubated at 32°C with shaking. At indicated times, 5 ml aliquots were taken out and induced at 42°C for 15 min and then incubated at 39°C. At different times, 0.05 ml aliquots were taken out from both 32°C (control) and 39°C (induced) sets and pulsed with 0.5 \( \mu Ci \) of [\(^{3}H\)] thymidine (17.2 Ci/mmol) for 3 min at the respective temperatures of their immediate previous growth. The TCA-insoluble radioactivity were then determined. At different times, viable cell numbers were determined from the set continuously growing at 32°C. 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.
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 594rpl8 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 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 Dral, EcoRI, EcoRV, PvuI, and PvuII (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 in the dnaA gene in a plasmid (see Methods). Three plasmids, pDMrpl10, pDMrpl12 and pDMrpl14 were obtained, which protected the wild-type

| Table 1. The map position of rpl mutation in the E. coli chromosome |
|---------------------------------------------------------------|
| Recipient          | From rpl     | Map       | Cotransduction | Approximate |
| E. coli strain     | donor        | position  | frequency      | map position|
| dnaAts46           | dnaA+        | 83 min    | 90/96 (94%)    | 83 min     |
| dnaBts             | dnaB+        | 92 min    | 0/50           | ----       |
| dnaCts             | dnaC+        | 99 min    | 0/50           | ----       |

For details, see Methods.

| Table 2. Lambda P-resistance property of different plasmids |
|-------------------------------------------------------------|
| Bacteria         | Survival after transformation with pMR45 DNAa |
|                  | CFU/mg of plasmid DNA % of control |
| 594 rpl8         | 9.03×10⁷         | 100 |
| 594 (wild type)  | <3.00×10⁷        | <0.03 |
| 594 (pLAR13)     | <3.00×10⁷        | <0.03 |
| 594 (pIDE2)      | <3.00×10⁷        | <0.03 |
| 594 (pDMrpl8-1)  | 9.00×10⁷         | 99.5 |
| 594 (pDMrpl8-2)  | 9.69×10⁷         | 107 |
| 594 (pDMrpl10)   | 9.03×10⁷         | 100 |
| 594 (pDMrpl12)   | 8.20×10⁷         | 90.8 |
| 594 (pDMrpl14)   | 8.20×10⁷         | 90.8 |

aAll the plasmids had cat marker. The bacteria (CaCl₂-competent) under first column were transformed with about 100 ng pMR45 DNA (Am²). Transformed 594rpl8 and 594 were plated on Amp plate and all others on Amp+Cm plates all at 37°C. The 594rpl8 bacteria were used as the P-resistant control.
Table 3 Complementation of dnaAts46 mutation by different plasmids

| Recipient bacterium | Transforming plasmid | Survival at 42°C after transformation (% of control at 32°C) |
|---------------------|----------------------|-------------------------------------------------------------|
| E. coli dnaAts46    | None                 | <0.07                                                       |
|                     | pLAR13               | 97.1                                                        |
|                     | pDE2                 | <0.07                                                       |
|                     | pDMrpl8-1            | 98.3                                                        |
|                     | pDMrpl8-2            | 97.1                                                        |
|                     | pDMrpl10             | 97.5                                                        |
|                     | pDMrpl12             | 98.5                                                        |
|                     | pDMrpl14             | 114                                                         |

E. coli dnaAts46 was grown at 32°C and transformed with different plasmids and grown, and plated in duplicates. One from each set was incubated at 32°C and the other at 42°C. The colony counts of the plasmid transformants for all the 32°C sets were around 5.5×10⁶ CFU/mg plasmid DNA. For other details, see Methods.

Fig. 3. Southern blot of the rpl clones digested with EcoRI and EcoRV and probed with [³²P]-labelled EcoRI-EcoRV 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, X/HindIII; 2, pDE2; 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.

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.

E. coli from λ P gene-induced killing (Table 2, lines 7-9) and complemented the E. coli dnaAts46 mutant at 42°C (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 from Ser (S) to Thr (T) respectively in the DnaA protein (the DnaA protein will be called as DnaC 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 oriC (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 oriC site, and P brings and loads DnaB onto the oriC-bound O protein to form the preprimosome (Dodson et al., 1989; Liberek 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 DnaA is lethal to host. The three rpl mutations have been located in the segment from 200 to 313 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 initiation 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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