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The Bacteriophage λ DNA Replication Protein P Inhibits the *oriC* DNA- and ATP-binding Functions of the DNA Replication Initiator Protein DnaA of *Escherichia coli*

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Under the condition of expression of λ P protein at lethal level, the *oriC* DNA-binding activity is significantly affected in wild-type *E. coli* but not in the *rpl* mutant. In purified system, the λ P protein inhibits the binding of both *oriC* DNA and ATP to the wild-type DnaA protein but not to the *rpl* DnaA protein. We conclude that the λ P protein inhibits the binding of *oriC* DNA and ATP to the wild-type DnaA protein, which causes the inhibition of host DNA synthesis initiation that ultimately leads to bacterial death. A possible beneficial effect of this interaction of λ P protein with *E. coli* DNA initiator protein DnaA for phage DNA replication has been proposed.

Keywords: *dnaA* gene of *E. coli*, Lambda *P* gene, *oriC* DNA, λ P-DnaA interaction, λ *P* gene lethality

Introduction

Bacteriophages being obligate parasites exploit one or more of the gene functions of their host(s) for their own growth. Such exploitations imposed by the phages may be nonlethal or lethal to their host(s). The non-lethal interactions of bacteriophage λ genes with those of *Escherichia coli* have been studied in depth (Friedman *et al.*, 1984). These have been summarized in Datta *et al.* (2005) There are three reports of host toxicity by lambda. (1) Induction of λ*N*⁻*cIts* lysogen at 42°C causes bacterial killing that requires the phage replication genes *O* and *P* as well as the right promoter *p*R (Sly *et al*., 1968). (2) The *kil* gene located between the γ and *c*III genes in the left operon of λ has been reported to be lethal to *E. coli* (Greer, 1975a). The Kil protein possibly interacts with certain component (s) of the cell envelope (Greer, 1975b) and inhibits division (Sergueev *et al*., 2001) (3) The CII protein of this phage has been reported to inhibit host DNA replication (Kedzierska *et al*., 2003)

We showed for the first time that the DNA replication gene *P* of λ when expressed at elevated level caused bacterial killing even in the absence of phage DNA replication (Maiti *et al*., 1991a) and that the *E. coli rpl* mutants which were *r*esistant to λ *P* gene *l*ethality could be isolated by the mutagenesis of this bacterium (Maiti *et al*., 1991b), and also the *rpl*-like mutations have been isolated by *in vitro* mutagenesis of the *dnaA* gene is a plasmid (Datta *et al*., 2005). All those *rpl* mutations have been located within the bacterial DNA initiator gene *dnaA* (Datta *et al*., 2005). All these suggest that the λ *P* gene-mediated host lethality possibly involves its certain type of interaction with the DnaA protein (the DnaA protein will be called as DnaA in the text) of *E. coli*. In this paper we show by using purified proteins that the *oriC* DNA and ATP binding functions of DnaA are inhibited by the λ P protein (the λ P protein will be called as P in the text).

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), and tryptone agar and soft agar are described in Chattopadhyay and Mandal (1982), and those of Luria-Bertani (LB) broth in Sambrook *et al*. (1989). *E. coli* DH5 *(endI⁻r ⁻m⁺ suII⁺ thi⁻ recA gyr⁻ rel*) was obtained from S. Adhya. The lysogens *E. coli* 594 (λ112) and 594*rpl8* (λ112) (Datta *et al*., 2004) and λ*N*⁻*cI*⁻*hk* phage (Maiti *et al*., 1991a) were used from laboratory stocks.

E. coli cultures were routinely grown in TB, TBM (for λ infection experiments) or LB at 37°C with shaking, and the growth

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was monitored by measuring the OD of the culture at 590 nm. The bacteria harboring plasmid were grown in the presence of required antibiotic. Bacteriophage λ was prepared by growth and lysis in permissive *E. coli* in TBM. Plasmid DNA was isolated by the procedure as described (Sambrook *et al.*, 1989). The plasmid pTSO200 DNA (Oka *et al*., 1980) was labeled with [^{3}H] by growing the bacteria (harboring pTSO200) for 16 h in the the presence of Cm (15 µg/ml), [^{3}H]-thymidine (1 µCi/ml, 17.2 Ci/mmol) and 2-deoxyadenosine (250 µg/ml) in TB, and then the plasmid DNA was isolated.

Plasmids Recombinant DNA methods used were as described in Sambrook *et al*. (1989). The plasmid pALO12 contains the wild-type *dnaA* gene tagged to the *lacP* promoter (Lobner-Olesen *et al*., 1989). The plasmid pTSO200 (Oka *et al.*, 1980) contains the minimal 245 base pair (bp) *ori*C DNA sequence of *E. coli*. pSP562 (obtained from D. Chattoraj, NIH, USA) contains the wild-type *dnaB* gene of *E. coli* tagged to the *p*L promoter. The plasmid pMM3 is a *cro-t*R1-*c*II-*O* deletion derivative of pMR45 (Fig. 1, Maiti *et al*., 1991a). In the absence of *cro* and *t*R1, this plasmid expresses the *P* gene at an elevated level from the *p*R promoter in the absence of λ repressor. pDM1 (Fig. 1) is a λ *p*L-based expression plasmid and was made as follows: the 1.2 kb DNA segment flanked by the *Bam*HI site at the left of *N* and the *Bgl*II site at the left of *c*I was gel purified and ligated with *Bam*HI-digested 4.36 kb pBR322 DNA. The ligated plasmid pDM1 (5.56 kb) was selected by its Tet sensitivity and Amp resistance. Any gene cloned at the *Bam*HI site in the direction of *p*L promoter would be expressed in the absence of λ repressor. pDM2 (Fig. 1) was constructed by ligating the gel-purified *Bam*HI 1.5 kb DNA segment containing the wild-type *dnaA* gene from pLAR13 (Fig. 1) with *Bam*HI-digested pDM1. The plasmid pDM2 (7.06 kb) having the *E. coli dnaA* gene oriented in the direction of *p*L promoter was selected by its ability to complement *E. coli danAts46* mutant at 42ºC.

Transformation and λ P sensitivity of *E. coli* These were done by the procedures exactly as described in the preceding paper (Datta *et al*., 2005).

Purification of proteins and their functional assays Wild-type DnaA was purified from *E. coli* 594 (λ112) harboring pDM2 containing the wild-type *dnaA* gene tagged to the *p*L promoter (Fig. 1). The bacteria were grown at 32ºC to 0.4 $OD_{590,}$ induced at 42ºC for 15 min, and then grown further for 1 h at 38ºC. The cells were then harvested and used to purify the DnaA protein by the procedure of Sekimizu *et al.* (1988). The *rpl8* DnaA protein was purified from *E. coli* 594*rpl8* harboring pDMrpl8-1 (Fig. 1) also by the procedure of Sekimizu *et al*. (1988). During purification, the DnaA containing fractions were monitored by *oriC* DNA-binding assay as described later. The DnaB protein (this protein will be called as DnaB in the text) was partially (>1,000 fold) purified (through the streptomycin sulphate, ammonium sulphate and DEAE-Cellulose steps) from heat-induced *E. coli* 594 (λ112) harboring pSP562 by the method of Reha-Krantz and Hurwitz (1978a), and the fractions containing DnaB were monitored by determining ss-DNA-dependent ATPase activity (Reha-Krantz and Hurwitz 1978b).

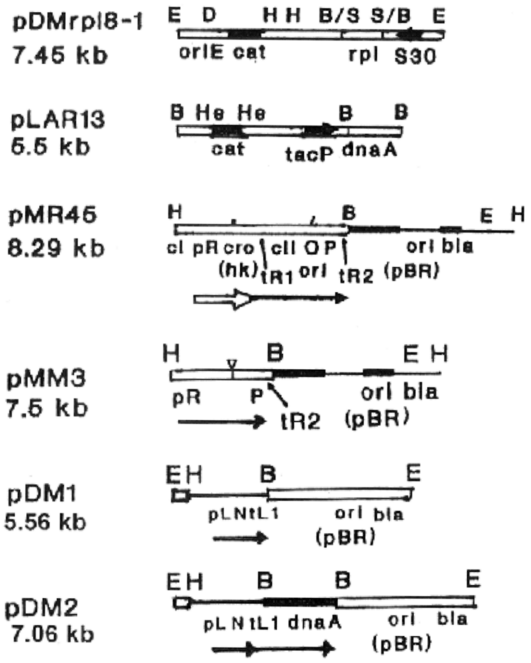

Fig. 1. Maps of different plasmids**.** Maps of the plasmids are shown in linearized forms nearly to the same scale. Sizes of the plasmids in kb are indicated by the numbers written below the names of the plasmids at the left. Approximate positions of the restriction enzyme cleavage sites are indicated 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 symbols: B, *Bam*HI; D, *Dra*I; E, *Eco*RI; and H, *Hin*dIII; S, *Sau*3AI.

The λ P protein was purified from *E. coli rpl8* (λ112) harboring pMM3 (Fig. 1). The bacteria were grown at 32ºC to 0.4 $OD_{590,}$ induced at 42ºC for 15 min and then grown for 1 h at 38ºC. The cells were then harvested. The P protein was isolated by the procedure of Tsurimoto *et al*. (1982). The fractions containing P was monitored by its ability to inhibit the ATPase activity of DnaB in the presence of ss-DNA (Reha-Krantz and Hurwitz, 1978b).

All the three protein preparations (wild-type DnaA, *rpl* DnaA and P) were more than 90% homogeneous. Both the purity and molecular weights of all these proteins were checked by SDS polyacrylamide gel electrophoresis (Sambrook *et al*., 1989).

The binding of DnaA to *oriC* DNA was measured by the filter-binding method (Fuller and Kornberg, 1983). The reaction mixture contained 40 mM HEPES-KOH (pH 7.6), 150 mM KCl, 10 mM Mg-acetate, 2 mM DTT, and 100 µg/ml of BSA, 150 ng of [^{3}H]-labeled pTSO200 plasmid DNA and the required amount of DnaA in a total volume of 25 µl. After incubation for 15 min at 30ºC, 20 µl of this reaction mixture was filtered on Millipore filter and washed with 40 mM HEPES buffer. The radioactive counts retained on the filter were determined.

The ATP-binding activity of DnaA was measured by the method of Sekimizu *et al*. (1987). The reaction mixture contained 50 mM HEPES-KOH (pH 8.0), 0.5 mM Mg-acetate, 0.3 mM EDTA, 5 mM DTT, 10 mM ammonium sulphate, 17% v/v glycerol and 0.005%

Triton X 100, 0.03 μM [α-^{32}P]ATP, and DnaA in a total volume of 40 μl. This mixture was incubated at 0°C for 15 min and then filtered on Millipore filter. In an alternate (modified) method, the reaction mixture (after incubation) was mixed with 160 μl cold saturated ammonium sulphate solution and kept on ice for 30 min. The precipitated protein with bound ATP was collected by centrifugation at 12,000 × *g* for 15 min at 4°C and washed once with 200 μl cold saturated ammonium sulphate solution. Finally, the pellet was dissolved in 200 μl sterile distilled water, and the radioactive counts were determined. Identical binding results were obtained when [γ-^{32}P]ATP was used in place of [α-^{32}P]ATP. This method was adapted from the one developed by Anderson *et al.* (1971) for the assay of the binding of cyclic AMP to CRP. Following this method, the ATP-DnaA binding results were very reproducible.

Radioactivity and protein measurements Radioactive counts were determined in a Beckman LS 5000CE counter. Protein was quantified by the method of Bradford (1976).

Results

The *oriC* DNA-binding activity in wild-type *E. coli* is affected after λ*N*⁻*cI*⁻*P*⁺*hk* phage infection At the time of initiation of chromosomal DNA replication in *E. coli*, the DnaA protein binds to several DnaA-specific conserved 9-mer boxes present within the *oriC* DNA (Fuller *et al*., 1984). This DNA-binding activity is an important property of DnaA and can be determined by Millipore filter-binding assay (Fuller and Kornberg, 1983). When a nonpermissive *E. coli* is infected with the λ*N*⁻*cI*⁻*P*⁺*hk* phage at a multiplicity of infection (MOI) of 50 or more, the bacterium is killed (Maiti *et al*., 1991a). To find out if there had been any loss of the *oriC* DNA-binding activity following challenge with P, we examined the effect of infection by λ*N*⁻*cI*⁻*P*⁺*hk* phage on the above activity in wild-type *E. coli*. The results presented in Table 1 show that the *oriC* DNA-binding activity was significantly reduced following infection of *E. coli* (producing wild-type DnaA) with the above phage. Under identical conditions, however, the *oriC* DNA-binding activities were not reduced at all in the λ*N*⁻*cI*⁻*P*⁻*hk* phage-infected wild-type bacteria and in both the above l*P*⁺ and l*P*⁻ phage-infected *rpl* bacteria (Table 1).

The λ P protein affects the *oriC* DNA-binding function of DnaA The reduction in the *oriC* DNA-binding activity in wild-type *E. coli* by λ*N*⁻*cI*⁻*P*⁺*hk* phage infection (Table 1) led us to ask as to whether P inhibits the above function of DnaA. To answer this question, we examined the effect of purified P on the *oriC* DNA-binding activity of purified DnaA. The results presented in Fig. 2 clearly reveal that when P was added to the reaction mixture before the addition of *oriC* DNA, the binding of wild-type DnaA to *oriC* DNA was inhibited, and the amount of inhibition increased with the amount of P added (curve A). This inhibition reached its maximum at around the P : DnaA weight ratio of 1 : 1 (at a subunit molar ratio of 2 : 1; the molecular weights of P and DnaA are 25.63 kDa and 51.37 kDa respectively). When *oriC* DNA was added to DnaA prior to the addition of P, there was no such inhibition (curve B). However, under identical conditions, the binding of *rpl* DnaA to *oriC* DNA was not inhibited when P was added to *rpl* DnaA both before (curve C) and after (curve D) the addition of *ori*C DNA (Fig. 2). It was found that the PCR amplified 110 bp DNA containing three conserved 9-mer DnaA boxes R2, R3, and R4 from the minimal 245 bp *oriC* region within the pTSO200 DNA (the minimal 245 bp *oriC* DNA contains five such DnaA boxes) could compete with radioactively labeled *oriC* plasmid DNA; while the fragment of pTSO200 DNA that did not contain the 245 bp *oriC* DNA, did not show any such competition (data not shown). This suggests that the above binding of DnaA was specific for the *oriC* segment within the pTSO200 DNA.

Table 1. Effect of λ*N*⁻*cI*⁻*hkP*⁺ infection on the *oriC* DNA-binding activity in wild-type and *rpl* mutant *E. coli*

E. coli extract[a]	*OriC* DNA-binding activity[b] (pmol DNA bound/mg protein)
1. Uninfected wild type	2.15 ± 0.36
2. λ*N*⁻*cI*⁻*hk P*⁺-infectd wild type	0.40 ± 0.11
3. λ*N*⁻*cI*⁻*hk P*⁻-infectd wild type	2.52 ± 0.06
4. Uninfected *rpl8*	1.60 ± 0.08
5. λ*N*⁻*cI*⁻*hk P*⁺-infectd *rpl8*	1.70 ± 0.16
6. λ*N*⁻*cI*⁻*hk P*⁻-infectd *rpl8*	2.06 ± 0.03

[a]*E. coli* BR1639 (pALO12) (wild-type *dnaA*) and *E. coli* 594*rpl8* (pDMrpl8-1) were grown in TBM (the former in the presence of 2 mM IPTG) both to around 0.5 OD_{590}. From each culture, 2×5 ml aliquots were separately infected with the two phages, and 1×5 ml aliquots were kept as uninfected control. All the aliquots were grown further for 1 h. The cells were harvested, washed and broken by sonication, and centrifuged at 100,000 g for 35 min. The supernatants were treated with 0.28 g/ml of $(NH_4)_2SO_4$ in cold. The precipitates were collected by centrifugation, dissolved in storage buffer and dialyzed, and used for the DNA-binding assay. The above partial purification eliminated almost all of the non-specific DNA binding activities as shown by the fact that the binding to radioactive *oriC* DNA was competed with cold *oriC* DNA but not with cold non *oriC* DNA.
[b]Results represent the averages of three independent experiments. For other details, see Methods.

The λ P protein also affects the ATP-binding function of DnaA The DnaA protein binds ATP, and this ATP-DnaA complex is the functional form that is essential for the opening of the 13-mer sequences within the *oriC* DNA during the initiation of DNA replication (Sekimizu *et al*., 1987). As P inhibits the interaction of DnaA with *oriC* DNA (Fig. 2), we also examined the effect of P on the binding of ATP to DnaA. The results in Table 2 show that when wild-type DnaA was incubated with P and then ATP was added, the binding of this

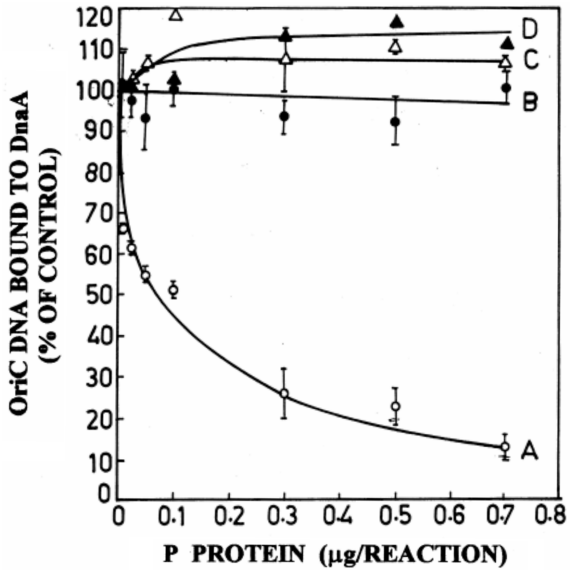

Fig. 2. Effect of λ P protein on the binding of *ori*C DNA to wild-type and *rpl8* DnaAs. The DNA-binding activity of DnaA was assayed as described in Methods. In *A* and *C*, the reaction mixtures containing P and DnaA were incubated for 10 min at 30°C and then *oriC* DNA was added and incubated further for 15 min at the same temperature. In *B* and *D*, DnaA was incubated with *ori*C DNA for 15 min and then P was added and incubated further for 10 min. The control (100%) values for the binding of *ori*C DNA to wild-type and *rpl*-type DnaAs were respectively 116 and 107 pmol/mg of protein. The amounts of DnaA and *ori*C DNA per reaction were used at 0.7 μg and 150 ng respectively (these amounts were determined to be required for maximum binding from saturation experiments). In control experiments, addition of varying amounts of P-storage buffer without P showed the pattern of binding of *oriC* DNA to wild-type and *rpl* DnaAs similar to that in B and D respectively. The results represent the averages of three experiments, and the standard deviations are shown. Curves: *A* and *B*, wild-type DnaA; and *C* and *D*, *rpl* DnaA. For other details, see Methods.

nucleotide to DnaA was drastically reduced (line 3). However, when DnaA was incubated with ATP and then P was added, the binding of the nucleotide was not affected (line 2). The interaction of ATP with *rpl* DnaA was not inhibited by P under any of the above conditions (Table 2, lines 4-6).

***E. coli dnaAcos* mutant is sensitive to λ *P* gene-mediated killing** The DnaAcos protein does not bind ATP but binds to *oriC* DNA and activates the initiation of DNA replication, though at 43°C (Katayama 1994). As the interaction of wild-type DnaA with both *oriC* DNA and ATP was inhibited by P, we were interested to see if the *E. coli dnaAcos* mutant is sensitive to P. The results in Table 3 show that the *E. coli dnaAcos* mutant was killed when challenged with P by transformation with pMR45. This suggests that P can interact with the mutationally altered DnaAcos protein (in the absence of ATP binding) and inhibits the binding of latter protein to *oriC* DNA thereby causing bacterial killing.

Table 2. Effect of the λ P protein on the binding of ATP to DnaA

DnaA type[a]	Ligand added[b]		nmol ATP bound[c] per mg of protein
	First	Second	
Wild type	ATP	None[d]	2.75 ± 0.05 (100)
	ATP	P	2.81 ± 0.11 (102)
	P	ATP	0.28 ± 0.005 (10.36)
rpl8 type	ATP	None[d]	1.82 ± 0.03 (100)
	ATP	P	1.86 ± 0.065 (102)
	P	ATP	1.89 ± 0.21 (113)

[a]Purified wild-type and *rpl8* DnaAs and P were used.
[b]After the addition of the first ligand, the reaction mixture was incubated for 15 min at 0°C, and then the second ligand was added, and incubated further for 10 min at 0°C. The reaction mixture without the second ligand was incubated for 25 min. The reaction mixture was mixed with required volume of cold saturated ammonium sulphate solution, and the protein-bound ATP in the precipitate was determined. The wild- and *rpl8*-type DnaAs and P were used at 0.7 μg each per reaction and ATP at 0.03 mM.
[c]The data represent the averages of two independent experiments. The figures in parenthesis indicate % of control.
[d]These reaction mixtures contained the volume of P-storage buffer without P that was equal to the volume of P added to the other reaction mixtures.
For other details, see Methods.

Discussion

The results presented in this paper show that the bacteriophage λ DNA replication protein P specifically inhibits the binding of *oriC* DNA and ATP.to wild-type DnaA but not to its *rpl* mutant form. This suggests that this inhibitory action of P on the binding of DnaA to *oriC* DNA and ATP causes a total arrest of host DNA synthesis, which ultimately leads to bacterial death. Thus the bacterial killing induced by P (which is not dependent on phage DNA replication, Maiti *et al*., 1991a) differs from that caused by the induction of λ*N*c*Its* lysogen at 42°C that requires phage DNA replication (Sly *et al*., 1968) and by the Kil protein of λ, which interacts with certain component(s) of cell envelope (Greer, 1975a; 1975b) and inhibits cell division (Sergueev *et al*., 2001).

The binding of DnaA to several 9-mer sequences within the *oriC* region is a prerequisite for the subsequent events that lead to the DNA replication initiation. The ATP-bound form of DnaA bound to *ori*C DNA helps open the 13-mer AT-rich sequence within this region to form the preprimosome, while the ADP-bound form can also bind to *oriC* DNA but fails to open the 13-mer AT-rich segment (Sekimizu *et al*., 1987). Both the expression (Messer and Weigel, 1997) and the activity of DnaA (Katayama *et al*., 1998; Sekimizu *et al*., 1987) are regulated in various ways to control its function related to the *oriC*-dependent initiation of DNA replication.

Table 3. Effect of the λ *P* gene product on the survival of *E. coli dnaAcos* mutant

Bacteria[a]	Plasmid[b]	Survival after transformation with different plasmids	
		CFU/mg DNA	% of Control
E. coli rpl8	pMR45	5.5×10^5	100
	pMR58	4.9×10^5	89.1
E. coli 594	pMR45	$<6 \times 10^2$	<0.1
	pMR58	5.2×10^5	94.5
E. coli dnaAcos	pMR45	$<6 \times 10^2$	<0.1
	pMR58	4.8×10^5	87.3

[a]After transformation, *E. coli rpl8* and 594 were plated at 37°C and *dnaAcos* at 42°C.
[b]The plasmid pMR45 is P^+ and pMR58 is P^-.
For other details, see Methods and Text.

We show that the function of DnaA is negatively regulated also by P by inhibiting the binding of both *oriC* DNA (Fig. 2) and ATP (Table 2) to DnaA.

The P protein inhibits the binding of *oriC* DNA to wild-type DnaA but not to *rpl* DnaA (Fig. 2). This inhibitory effect of P was observed only when this protein was added to wild-type DnaA before the addition of *oriC* DNA. This suggests that following the binding of *oriC* DNA with wild-type DnaA or due to the amino acid change(s) caused by the *rpl* mutation(s), the conformation of DnaA changes to certain form that does not allow its interaction with P. The data in Table 2 show that P also inhibits the binding of ATP to wild-type DnaA when P is added to DnaA before the addition of ATP. It was also observed that P inhibited the binding of *oriC* DNA to ATP-bound wild-type DnaA (our unpublished data). It is known that the DnaAcos mutant protein does not bind ATP, yet it can bind to *oriC* DNA and initiate DNA replication in the above mutant *E. coli* (Katayama, 1994). Our data show that the *dnaAcos* mutant bacterium is susceptible to P-induced killing (Table 3). If it is assumed that the *cos* mutant form of DnaA has a conformation similar to that of the ATP-bound form of the wild-type DnaA, then the above data suggest that the ATP-bound wild-type DnaA can bind to P, but following interaction with P, the wild-type DnaA does not bind ATP and/or *oriC* DNA.

DnaA is made up of 467 amino acid residues and has several functional domains (Sutton and Kaguni, 1997; Messer, 2002). Those domains of DnaA are involved in the binding of this protein to DNA (Roth and Messer, 1995), RepA protein (Sutton and Kaguni, 1995), ATP (Carr and Kaguni, 1996), DnaB (Marszalek *et al*., 1996), acidic membrane (Garner and Crooke, 1996) and in its self-oligomerization (Weigel *et al*., 1999). These domains are present in the above order from C to N termini of the DnaA protein (see Fig. 3). From this study, we define another domain called the P-interaction domain, which overlaps with the RepA protein-binding domain (Fig. 3). This domain of DnaA is flanked by the ATP-binding

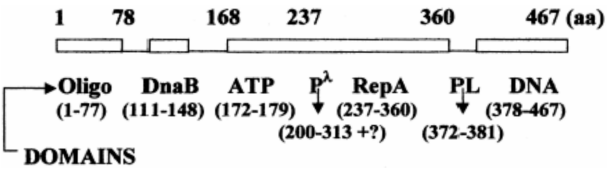

Fig. 3. The domain map of the DnaA protein. Approximate boundaries of different functional domains in the 467-residue DnaA protein are shown. The P interaction domain defined by the *rpl* mutations is shown (the boundary of this domain is only approximate, and this is indicated by ? sign). Different domain symbols: Oligomer, protein-protein interaction; DnaB, interaction with DnaB; ATP, binding of ATP; P^{λ}, interaction with λ P protein; RepA, interaction with RepA protein; PL, interaction with membrane phospholipid; and DNA, binding of *oriC* DNA

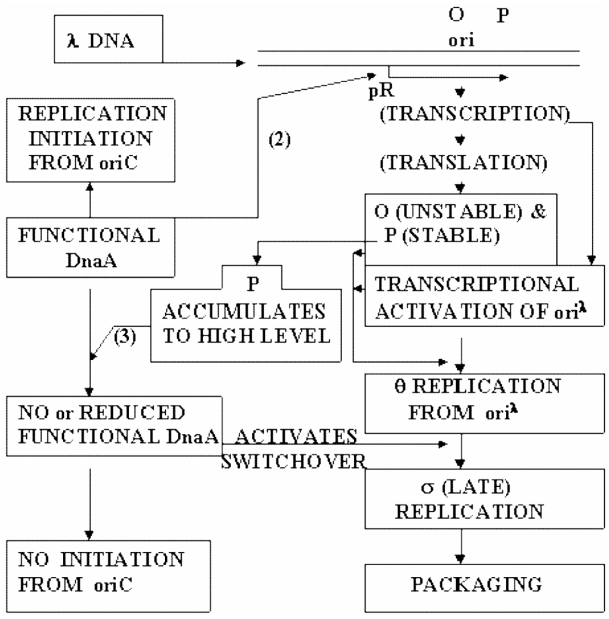

Fig. 4. Proposed model for the P-lethal circuit. (1) Activation of DNA replication initiation from *oriC* by DnaA; (2) Stimulation of transcription from *p*R of λ that leads to (a) increased level of expression of O and P and (b) transcriptional activation of ori^{λ} initiating theta replication; (3) The P protein at high level, inactivates DnaA, which (a) affects bacterial DNA replication initiation from *oriC* of *E. coli* and (b) activates the switchover from early (theta) to late (sigma) mode of λ DNA replication. For other details, see discussion.

domain at the N-terminal side and the DNA-binding domain at the C-terminal side. This, possibly, explains the fact that the binding of P to this region of DnaA inhibits the binding of both *oriC* DNA and ATP to the latter protein. However, the *rpl* mutational alteration in this domain, which confers on DnaA with its resistance to P lethality, does not affect the binding of either *oriC* DNA or ATP to *rpl* DnaA. The binding stoichiometry of 2 mole of P with one of DnaA in Fig. 2 may suggest that a dimer of P (Zylicz *et al*., 1984) possibly interacts with the above-defined P-interaction domain of

DnaA and disrupts the conformation/*orientation* of both the flanking ATP and DNA-binding domains (Erzberger *et al*., 2002) thereby disallowing the binding of ATP and *oriC* DNA to DnaA.

It may now be asked as to why the P protein of λ is involved in the inhibitory interaction with DnaA, and whether this interaction is beneficial for the growth and multiplication of λ. It has been reported by Konopa *et al.* (2000) that at 43ºC, the λ*Pts1piA66* mutant cannot replicate in wild-type *E. coli* but can do so in the *E. coli dnaAts46* mutant. The λ DNA replication has been shown to switch quickly from the theta to the sigma mode in the absence of functional DnaA (Konopa *et al*., 2000; Baranska *et al*., 2001). This also implies that DnaA possibly inhibits, directly or indirectly, the above switchover process. Therefore, our results along with those of Konopa *et al.* (2000*)* suggest that the interaction of P with DnaA has two effects: (a) it inhibits *E. coli* DNA initiation by inhibiting the binding of DnaA to *oriC* DNA and ATP, and (b) it helps λ replication to switchover from the theta to the sigma mode. The former effect appears to be lethal to the host cell, while the latter is beneficial to λ growth. DnaA also stimulates the transcription from *p*R of λ (Szalewaska-Palasz *et al*., 1998), thereby stimulating the expression of *P* (and *O*) genes and effecting transcriptional activation of ori^{λ}. The P protein is stable and is required only during the initiation of theta replication of phage DNA (Klinkert and Klein, 1979), while DnaA inhibits the theta to sigma switchover (Konopa *et al*., 2000; Baranska *et al*., 2001). So, in the above background, we propose that the DnaA protein of *E. coli* is used by λ for stimulating the initiation of circle to circle replication of its DNA by activating the expression of P (and O) from *p*R as well as by increasing the frequency of transcriptional activation of ori^{λ} at the early stage of phage growth. At the late stage, the accumulated (stable) P inactivates DnaA to facilitate switchover from the theta to the sigma mode of λ DNA replication possibly by reducing the frequencies of both transcription from *p*R and transcriptional activation of ori^{λ} (see Fig. 4 for the proposed model). By this process, the DnaA function is inhibited (which is lethal to host), and also the P protein, which is no longer required during the rolling circle replication of λ DNA, is removed or inactivated.

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