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A New Bacterial Gene (*groPC*) which Affects λ DNA Replication

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Summary. A bacterial mutation affecting λ DNA replication, called *groPC756*, has been mapped between the *thr* and *leu* bacterial loci. Most of the parental λ DNA does not undergo even one round of replication in this host. Lambda mutants, called π, which map in the λ *P* gene are able to overcome the inhibitory effect of the *groPC756* mutation. It is shown that the mutation at the *groPC* locus also interferes with bacterial growth at 42° C. A λ-transducing phage, carrying the *groPC*$^+$ allele, was isolated as a plaque-former on *groPC756* bacteria. Upon lysogenization, it restores both the *gro*$^+$ and temperature resistant phenotypes.

Introduction

Successful DNA replication of bacteriophage λ in *Escherichia coli* requires both phage and bacterial gene functions. The products of genes *O* and *P* are the only λ genes known to be required for λ DNA replication; mutants defective in either gene are unable to carry out even one round of DNA replication (Joyner et al., 1966; Ogawa and Tomizawa, 1968). It has been suggested that genes *O* and *P* code for an endonucleolytic nicking activity (Shuster and Weissbach, 1969), that their products interact to form a complex (Tomizawa, 1971) and that they are both needed for the synthesis of a small RNA transcript, called *oop*, implicated in the initiation of λ DNA synthesis (Hayes and Szybalski, 1973). Among the *dna* gene products necessary for *E. coli* DNA replication those of *dnaB, dnaE, dnaG* and *dnaZ* are required for λ DNA replication (Hirota et al., 1968; Kohiyama, 1968; Fangman and Feiss, 1969; Gross, 1972; Shizuya and Richardson, 1974; Walker et al., 1976).

We have previously reported the isolation and partial characterization of a class of bacterial mutants, called *groP*, which appear to block phage λ development at the step of λ DNA synthesis (Georgopoulos and Herskowitz, 1971). The majority of these mutants have been shown to map near *malB* and appear to be located in the *dnaB* locus of *E. coli* (Herskowitz, Georgopoulos and Kühnlein, manuscript in preparation; d'Ari et al., 1975). Here, I report on the characterization of another class of *groP* mutants, exemplified by *groPC756*, which appear distinct from those which map in *dnaB*. A bacterial mutant, called *groPC259*, which blocks both λ DNA replication and P2 phage propagation and maps very near *groPC756* is reported in this issue by Sunshine et al. Saito and Uchida (J. molec. Biol. in press and personal communication) have recently reported the isolation of new bacterial mutants which affect λ DNA replication. One class of these mutants maps in the same position as *groPC756*.

Methods and Materials

Bacterial and Phage Strains. Most of the bacterial and phage strains used in this study have been described before (Georgopoulos, 1971; Georgopoulos and Herskowitz, 1971). LC462 *serB thy*, LC37 C600 *leu thr*, LC302 F$^-$ *leu purE trp his metA argG proC araB lac xyl mtl gal* and LC164 *gal*$^-$/F′*gal*$^+$ were provided by Lucien Caro.

A pool of λ*imm*^{21} phage no. 540 carrying various restriction fragments of *E. coli* DNA (prepared by cleaving both phage no. 540 and *E. coli* DNAs by the R.*Hind*III restriction enzyme, annealing the fragments with ligase and selecting for plaque forming units) was kindly provided by Ken Murray (Murray and Murray, 1975).

Media, Bacterial and Phage Platings were as previously described (Georgopoulos, 1971; Georgopoulos and Herskowitz, 1971).

Isolation of groPC756. The isolation of the bacterial mutant B178 *groPC756* which is unable to propagate lambdoid phages has previously been described (Georgopoulos and Herskowitz, 1971).

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Transductions by Phage P1, F′ strain Construction and Bacterial Matings were carried out as described by Miller (1972).

Preparation of λ Phage Carrying BudR-substituted, ^{14}C-labeled DNA. A culture of C600 *thy* (*λimm*^{λ}*c*I857*Sam7*) was grown at 30° in M9 medium (Champe and Benzer, 1962) supplemented with 0.2% casamino acids, 0.2% glucose and 20 μg/ml thymine to about 2×10^8 cells/ml. The culture was washed twice with unsupplemented M9 medium. After a period of 10 min of thymine starvation at 30°C, BudR and ^{14}C-TdR were added at 20 μg/ml and 0.5 μg/ml (10 μC/ml) respectively. The culture was kept at 42°C for ten minutes and then shifted to 37°C for an additional two hours. The cells were collected by centrifugation and lysed with chloroform. After a five minutes centrifugation at 10,000 g to remove bacterial debris, the supernatant was treated with 5 μg/ml of Dnase I for 10 min at 37°C. Phage was purified first in a CsCl block gradient followed by two CsCl equilibrium centrifugations.

Density Shift Experiment. B178 *gro*$^{+}$ and B178 *groP*C756 bacteria growing exponentially in Tryptone broth were centrifuged and concentrated in 10^{-2} M $MgSO_4$ at 2×10^9 cells/ml. BudR-substituted, ^{14}C-labeled phage, prepared as described above, was added at a multiplicity of 0.5 phage per cell. Unadsorbed phage were removed by centrifugation, the infected cells were resuspended in prewarmed Tryptone broth at 1×10^8 cells/ml and shaken at 38.5°C for thirty minutes. The infected cells were collected by centrifugation and total DNA was extracted by the lysozyme–SDS procedure described by Bode and Kaiser (1965). A solution of saturated CsCl was used to adjust the density of the DNA preparations to approximately 1.745 g/ml followed by centrifugation in a Ti50 rotor at 38,000 rpm for 24 h. Each gradient was collected from the bottom into fity fractions and every other fraction was counted.

Results

*Characterization of groP756. GroP*756 (previously designated *groP*AB756, Georgopoulos and Herskowitz, 1971) belongs to the *groP* class of bacterial mutants which were isolated as large colony formers in the presence of phage *λimm*^{λ}*c*I*h*^{λ} and *λimm*^{434}*c*I*h*^{434} (Georgopoulos, 1971; Georgopoulos and Herskowitz, 1971). It does not allow the propagation of most lambdoid phages (except 424) but does allow the growth of non-lambdoid heterologous phages (such as P1, T4, T5, etc.). The block exerted by *groP*C756 bacteria on λ growth had previously been shown to be past the steps of phage adsorption and DNA penetration into the cell cytoplasm and most probably at the level of phage DNA replication. This was done in an indirect way by measuring the ratio of parental, P1-modified, DNA strands to newly synthesized, P1-unmodified DNA strands in the phage progeny (Georgopoulos and Herskowitz, 1971). A more direct experiment which shows that phage λ DNA replication is indeed blocked on *groP*C756 bacteria is shown in Figure 1. *gro*$^{+}$ and *groP*C756 bacteria were infected with λ phage whose DNA was doubly labeled with ^{14}C- and BudR. After minutes at 38.5°C total DNA was extracted from the infected cells and banded in

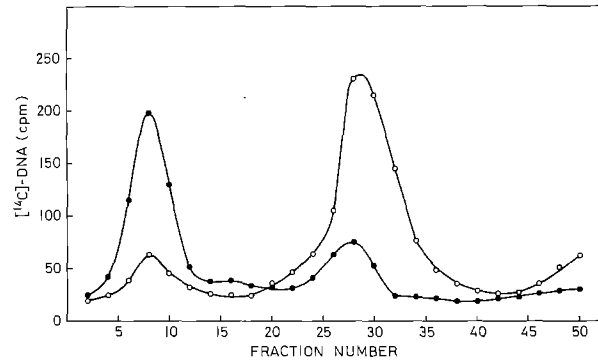

Fig. 1. CsCl equilibrium density centrifugation of DNA extracted from B178 *gro*$^{+}$ –o–o– and –•–•– B178 *groP*C756 bacteria infected at 38.5° C for thirty minutes in Tryptone broth with *λimm*^{λ}-*c*I857*Sam7* phage carrying BudR-substituted, ^{14}C-labeled DNA. The positions of the two peaks correspond to densities of 1.7903 for that of the heavy fraction and 1.7474 for that of the hybrid fraction

a CsCl equilibrium gradient. If the parental phage DNA replicates even once during the time of infection one would expect to find the radioactivity at the position of DNA with hybrid density. This was indeed found to be the case during infection of the *gro*$^{+}$ parental strain. Over eighty-five percent of the radioactivity was found at the position of hybrid density DNA (Fig. 1). The opposite result was found during infection of *groP*C756 with over seventy percent of the radioactivity associated with the heavy, parental DNA molecules. This result suggests that the majority of the parental λ DNA molecules did not even undergo a single round of DNA replication on *groP*C756 bacteria during the time of infection.

Phage Mutants (π) which Plate on groPC756. Another indirect observation which suggested that λ DNA replication is affected on *groP* hosts was the isolation of λ phage mutants which mapped in or around gene *P* and could overcome the block exerted by the host. These phage mutants, called π, were used to arbitrarily divide our *groP* mutants into two classes, *groP*A and *groP*B (Georgopoulos and Herskowitz, 1971). Lambda mutants called πA, isolated as plaque-formers on *groP*A hosts were unable to propagate on *groP*B bacteria. The reverse phenomenon was not true, inasmuch as lambda mutants called πB, isolated on *groP*B hosts, were able to plate on all *groP* bacteria (Table 1). The exception to this rule was found to be *groP*C756 (and hence the reason why it was originally called *groP*AB756) inasmuch as fifteen percent (15/100) of the π mutants isolated on this host plated on *groP*B558 bacteria whereas ninety-nine percent (99/100) plated on *groP*A15 and only five percent (5/100) plated on *groP*C259 bacteria (Sunshine et al.,

Table 1. Efficiency of plating[a] of $\lambda\pi$ mutants

Bacterial strain	λcI	$\lambda cI\pi$A1[b]	$\lambda cI\pi$B1[b]	$\lambda cI\pi$C2[c]	$\lambda cI\pi$C3[c]	$\lambda cI\pi$C4[c]
C600 *gro*$^+$	1.0	1.0	1.0	1.0	1.0	1.0
C600 *groP*C756	2.2×10^{-6}	1.5×10^{-3}	1.0	0.38	0.63	0.32
C600 *gro*PA15	7.1×10^{-6}	0.75	1.0	0.05	1.25	1.0
B178 *gro*PB558	$<5\times10^{-8}$	1.0×10^{-7}	0.45	1.5×10^{-7}	$<5\times10^{-8}$	0.28

[a] Efficiency of plating denotes the number of plaques produced by a phage strain on a given bacterial host at 37° C relative to the number on B178 *gro*$^+$ bacteria

[b] $\lambda cI\pi$A1 was isolated as a plaque-former on *groP*A15 was $\lambda cI\pi$B1 was isolated as a plaque-former on *groP*B558 (Georgopoulos and Herskowitz, 1971)

[c] Isolated as plaque formers on *groP*C756

Table 2. P1 transduction studies with *gro*PC756

Donor	Recipient	Selected Markers	Unselected Markers	Frequency
1. *thr*$^+$–*leu*$^+$–*groP*C756	*thr*$^-$*leu*$^-$*gro*$^+$ (LC37)	*thr*$^+$	*groP*C756	129/250
			gro$^+$	121/250
		leu$^+$	*groP*C756	3/304
			gro$^+$	301/304
		thr$^+$ *leu*$^+$	*proP*C756	78/97
			gro$^+$	19/97
2. *serB*$^+$ *thr*$^-$ *groP*C756	*serB*$^-$ *thr*$^+$ *gro*$^+$ (LC462)	*serB*$^+$	*thr*$^+$ *groP*C756	5/103
			thr$^-$ *groP*C756	25/103
			thr$^+$ *gro*$^+$	46/103
			thr$^-$ *gro*	27/103
3. Suggested Genetic order:		*ser B thr groP*C756 *leu*		

this issue). The plating properties of 5 typical λ π mutants are given in Table 1. In our previous publication, we reported that a variety of *Pam* mutants can plate on *groP*A15*su*$^+$II bacteria (Georgopoulos and Herskowitz, 1971). None of these *Pam* mutants was found able to plate on *groP*C756*su*$^+$II.

Mapping of groPC756. The majority of the *groP*A and *groP*B mutants in our collection which have been mapped were shown to be approximately sixty percent co-transducible by phage P1 with *malB* and appear to be located in the *dnaB* locus of *E. coli* (Herskowitz, Georgopoulos and Kühnlein, manuscript in preparation; d'Ari et al., 1975). *GroP*C756 was the only *groP* mutant mapped which did not co-transduce with *malB*. The approximate map location of *groP*C756 was determined as follows: the strain B178*groP*C-756*gal*$^-$/F'*gal*$^+$ was constructed and mated with LC102, a multiple auxotrophic female strain. Recombinant clones for various bacterial markers were scored for possession of the *groP* phenotype. It was found that the *groP* character was co-transferable at a high frequency with the *leu* locus. More precise mapping by P1 transduction showed *groP*C756 to be about fifty percent co-transducible with *thr*. Two- and three-factor crosses, as those reported in Table 2, established the genetic order *serB*—*thr*—*groP*C756—*leu*. From this genetic analysis it appears that the locus defined by *groP*C756 is distinct from the *dnaC(D)* locus of *E. coli* which maps to the left of *serB*.

Bacterial Phenotype of the groPC756 Mutation. *GroP*C756 bacteria were isolated at 30° C on the basis of their ability to propagate in the presence of lambdoid phages (Georgopoulos, 1971). In spite of the fact that no other selection was exerted, after subsequent testing it was found that the *groP*C756 bacteria were unable to form colonies at 42° C. Figure 2 shows the effect of a temperature shift (30° to 43° C) on the growth of *groP*C756 bacteria. It can be seen that after about two doublings at the high temperature the bacterial culture stops growing as judged both by viable count measurements and increase in the optical density. Preliminary experiments suggest that at the time of arrest of cell division the rate of DNA synthesis declines faster than the rate of RNA synthesis (Georgopoulos, unpublished data).

The groPC756 Mutation is Responsible for the Bacterial Temperature Sensitive Phenotype. In order to prove that the bacterial temperature sensitivity of

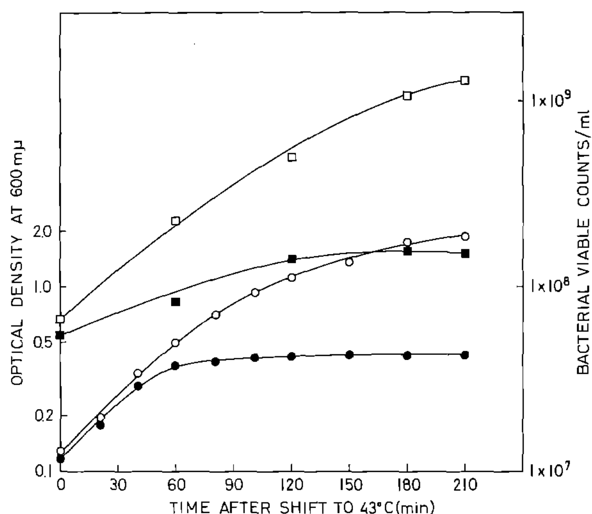

Fig. 2. Bacterial growth of C600 *thr⁻leu⁺ groPC756* and C600 *thr⁻leu⁺ gro⁺* in M9 medium supplemented with 0.2% casamino acids and 0.2% glucose. Bacterial cultures, pregrown at 30°C, were shifted to 43°C at t=0 min. Optical density at 600 mμ of *gro⁺* –o–o– and *groPC756* –●–●–. Bacterial viable counts, assayed at 30°C, of *gro⁺* –□–□– and *groPC756* –■–■–

strain *groPC756* is due to the *groPC756* mutation itself and is not a secondary consequence of the nitrosoguanidine mutagenesis used in the original selection, two approaches were used. Firstly, the co-transduction of the bacterial temperature sensitivity and the *groP* phenotype was tested in the P1 transduction studies shown in Table 2. In all cases examined it was found that the two traits were always co-transducible. In particular, all (129/129) of the *thr⁺groPC756* transductants of cross 1, Table 2, were shown to have simultaneously become temperature sensitive for growth at 42°C, whereas none (0/121) of the *thr⁺gro⁺* transductants were found to be temperature sensitive for growth at 42°C. This result suggests that both phenotypes are due to one mutation although it does not exclude the possibility of two closely linked mutations. Secondly, temperature resistant bacterial revertants of strain C600 *groPC756* were isolated at 43.5°C at a frequency of 5×10^{-8}, the majority of which had simultaneously lost their *groPC* phenotype, again suggesting that the *groPC756* mutation alone is responsible for both phenotypes. Interestingly, the majority of temperature resistant bacterial revertants isolated at 42° at a frequency of 1×10^{-7} retained their *groP* phenotype. These revertants are unable to grow at 43.5°C and probably represent some type of intragenic suppressor mutations. This was verified by P1 transduction studies which showed that the original *groPC756* mutation and the one causing the leaky temperature resistant phenotype could not be separated, suggesting that they are very closely linked indeed.

Isolation of λ Transducing Phage Carrying the groPC⁺ Bacteria Gene. A transducing phage carrying the *groPC⁺* bacterial gene was isolated as a plaque former on *groPC756* bacteria by plating a pool of λimm^{21} derivative phages carrying various segments of the bacterial chromosome (Murray and Murray, 1975). The frequency of isolation was 4×10^{-4}, about 200-fold higher than the occurrence of λ πC mutants. An isolate, designated $\lambda imm^{21}(groPC)^{+}$, was analyzed further. It was found that unlike the majority of λ πC mutants it did not propagate on *groPA15* bacteria, a property expected of a $(groPC)^{+}$ transducing phage but not of λπC: a *groPC756* host lysogenic for λimm^{21} $(groPC)^{+}$ becomes both temperature resistant and able to propagate λimm^{λ} phage, suggesting that the complete *gro⁺* phenotype has been restored. These observations suggest that the bacterial segment carrying the $(groPC)^{+}$ gene on the phage chromosome is not subject to phage repressor control i.e. it probably possesses its own promotor.

Discussion

The results presented in this paper demonstrate the existence of a new bacterial locus, defined by the *groPC756* mutation, which affects bacteriophage λ DNA replication. The *groPC* locus maps between the *thr* and *leu* loci and therefore appears to be different from the known *dna* bacterial genes which affect host DNA replication.

The mutation *groPC756* blocks λ replication as judged by two criteria: 1. A high proportion of the progeny phage retains the DNA modification of the parental phage (Georgopoulos and Herskowitz, 1971), and 2. Bu-labeled phage DNA fails to convert to hybrid density following infection of *groPC756* (Fig. 1). A role for the *groPC* gene in λ DNA replication is also indicated by the discovery of a class of phage mutants in gene P, called π, which are able to grow both on the *groPC756* host and on the *groPA* and *groPB* mutants which map in the *dnaB* locus (Herskowitz, Georgopoulos and Kühnlein, manuscript in preparation). These results, combined with those of Wickner and Hurwitz (1975) which show a functional interaction of the *dnaB* and *C(D)* products, suggest that λ DNA replication requires an enzyme complex containing (at least) the *dnaB*, *dnaC(D)* and *groPC* bacterial gene products and the phage gene *P* product. Other explanations have also been considered (Georgopoulos and Herskowitz, 1971).

In addition to blocking λ DNA replication, the *groPC756* mutation also interferes with bacterial growth at 42°C. After two doublings at the nonpermissive temperature, bacterial growth is arrested

(Fig. 2). In this respect *groP*C756 does not behave similarly to the *groP*A or *groP*B strains or to the known *dna* mutants of *E. coli* whose cell mass continues to increase at the non-permissive temperature even after arrest of DNA synthesis, with the consequent formation of long bacterial filaments (Hirota et al., 1968; Georgopoulos and Herskowitz, 1971). Preliminary experiments suggest that bacterial DNA synthesis is affected to a greater extent than RNA synthesis in *groP*C756 cultures at 43° C at the time when the optical density remains constant (Fig. 2). It remains to be shown, however, whether the primary effect of the *groP*C756 mutation on bacterial metabolism is exerted at the level of DNA replication.

The *groP*C756 mutation appears to be closely linked to the *groP*C239 mutation (Sunshine et al., this issue) and to the *dnaK* group isolated by Saito and Uchida (J. molec. Biol. in press and personal communication). It is not clear, however, if these mutations are allelic. The fact that the λimm^{21} $(groPC)^{+}$ transducing phage isolated as a plaque former on *groP*C756 does not propagate on *groP*C239 suggests that the *groP*C756 and *groP*C259 mutations may indeed belong to different bacterial cistrons.

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