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PROPHAGE P2 DOES NOT KILL recB BACTERIA

SHOJI SHINOMIYA and YOSHIYUKI SAKAKI*

Institute of Applied Microbiology, University of Tokyo, Yayoi, Bunkyo, Tokyo and Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan

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Summary: Escherichia coli carrying a temperature-sensitive recB mutation and lysogenic for phage P2 was able to grow normally even at 42°C, at which temperature the bacteria are phenotypically $recB^-$. At this temperature, the bacteria were, however, unable to support the growth of λspi^- phages.

INTRODUCTION

Bacteriophage P2 is unable to lysogenize recombination-deficient bacteria of the $recB^-$ or $recC^-$ classes, but P2 phage which is mutated in its old gene can lysogenize these bacteria (1). Based on the observations, Sironi (1) proposed that the old^+ gene product which is produced by prophage P2 kills $recB^-$ or $recC^-$ bacteria. This hypothesis was supported by Lindahl et al. (2) who studied the effect of the old^+ gene product on $recB^-$ bacteria using P2 phage carrying a mutation in its gene B, a gene for DNA synthesis and host-killing ability. They found that P2 B^-old^+ phage does not normally kill rec^+ bacteria because of the absence of functional gene B but does kill $recB^-$ bacteria. The $recB^-$ bacteria, however, remain viable if a P2 B^-old^- mutant is used. It was also found that the infection of P2 old^+ phage on $recB^-$ hosts causes the inhibition of protein, RNA and DNA synthesis (3). The old^+ gene product of phage P2 also has an inhibitory effect on the growth of phage λ (2-4). Since the gam gene of λ which plays important roles in this "P2-λ

Present address: *Department of Biochemistry, School of Medicine, Kyushu University, Fukuoka 812, Japan

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interference" phenomenon (4) produces an inhibitor of the host RecBC DNase and renders the host phenotypically recB$^-$ (5-7), the inhibitory effect of the old$^+$ gene product for λ has been formally understood through the killing effect of old$^+$ gene product on recB$^-$ hosts.

Thus, the P2 old$^+$ gene product appears to have a lethal effect on recB$^-$ bacteria, but it is still unknown whether the P2 old$^+$ gene product by itself kills recB$^-$ bacteria or other phage genes also participate in this host killing process, because experiments mentioned above cannot completely exclude the latter possibility. For further understanding the mechanism of the killing of recB$^-$ bacteria by P2 old$^+$ phage, we ask, at first, whether the P2 old$^+$ gene product is the only requirement for killing recB$^-$ bacteria. In this paper, we show that the presence of P2 old$^+$ gene product in recB$^-$ cells does not have any effect on macromolecule synthesis and cell viability.

MATERIALS AND METHODS

Bacterial and phage strain: Escherichia coli N138 has a temperature-sensitive recB mutation, recBts1 (8). This temperature-sensitive recB mutation was also introduced into Escherichia coli KMBL82PE1 (9) by P1 transduction. λcI857 reverse is a mutant phage which is insensitive to P2 interference (10). λcI857 bio10 and λcI857bio1 are deletion-addition mutants which have deleted most of the λ recombination region including gam gene (11). Phage P2 was kindly provided by Dr. R. Calendar.

Media: Otherwise noted, bacteria and phages were grown in L-broth containing 1% Bacto-tryptone, 0.5% yeast extract and 0.5% NaCl. Top and bottom agar contain 0.5% and 1.2% agar, respectively. M9-casamino acid medium contains 5.8 g of $NaHPO_4$, 3 g of KH_2PO_4, 5 g of NaCl, 1 g of NH_4Cl, 4 g of glucose 1 ml of 0.1 M $CaCl_2$, 10 ml of 0.1 M $MgCl_2$, 1 ml of 1 mM $FeCl_3$ and 5 g of Casamino acid per liter.

Isolation of P2 lysogens carrying recBts mutation: Bacteria were grown in L-broth and spread on L-broth agar plate with P2 phage to form plaques. After incubation at 33°C overnight, bacteria at the center of plaque were picked up with tooth pick, spread on L-broth plates, and grown at 33°C. Each colony was tested for phage sensitivity. Bacteria were isolated which are sensitive to λreverse but insensitive to wild type λ and P2. The presence of the temperature-sensitive recB mutation was confirmed by measuring the ATP-dependent DNase activity at 30°C and 40°C with the method previously described (12).

Macromolecule biosynthesis: Bacteria were grown in M9-casamino acid medium to the middle of the logarithmic phase. Then, macromolecule biosynthesis was followed by the incorporation of radioactive precursors to acid-insoluble

fraction. [^{14}C] amino acid mixture (final concentration of ~ 0.1 μC/ml), [^{3}H]uracil (4 μCi/ml) and [^{3}H]thymidine (10 μCi/ml) were used as precursors for synthesis of protein, RNA and DNA, respectively. In the case of DNA synthesis, 0.5 μg/ml of thymidine and 50 μg/ml of 2-deoxyadenosine were also added at the same time.

RESULTS AND DISCUSSION

Since recB$^-$ cells cannot be lysogenized by phage P2, we constructed P2 lysogen carrying a temperature-sensitive recB mutation to see the effect of prophage P2 on recB$^-$ cells. This P2 lysogen allowed us to investigate the effects of the P2 old$^+$ gene product, which is produced by prophage, on recB$^-$ bacteria by raising the temperature without any effects of other phage functions which may be expressed after the infection of the phage. In the prophage state, the two other genes, imm and fun, are also expressed but not involved in the killing of recB$^-$ bacteria (13).

The growth of this bacteria was studied at 42°C, at which temperature the bacteria are phenotypically recB$^-$. As shown in Fig. 1, N138recBts(P2) was able to grow normally at 42°C as well as N138recBts non-lysogenic for P2. N138recBts(P2) made normal-size colonies at 42°C and the plating efficiency at 42°C was about 0.8. The bacteria which survived at 42°C were not rec$^+$ revertants or bacteria cured of phage P2, because they were still as sensitive to ultraviolet light at 42°C as N138recBts and phage P2 and wild type λ did not plate on the survivors. Synthesis of protein, RNA and DNA in N138recBts (P2) was followed by the incorporation of radioactive amino acids, uracil and thymidine into acid-insoluble fractions, and no significant difference was observed between N138recBts(P2) and N138recBts at 42°C (Fig. 2). It is unlikely that the phenomena described above are specific for N138 strain, because the P2 lysogen of E. coli KMBL82MPE1recBts which has a different genetic background from N138 was also able to grow normally at 42°C (data are not shown). Thus, the presence of prophage P2 in recB$^-$ cells does not affect the cell viability or macromolecule synthesis.

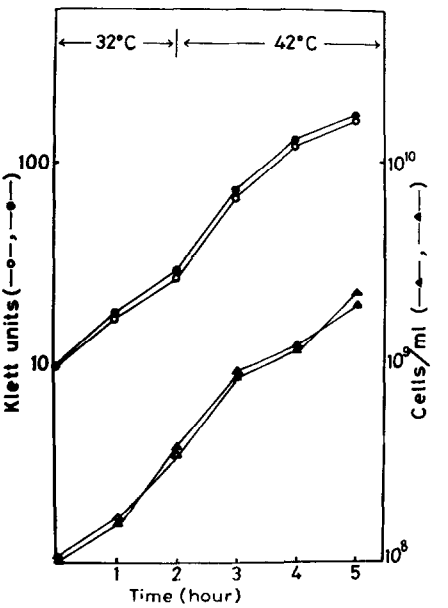

Fig. 1. Growth of N138recBts and its P2 lysogen. Bacteria were grown in L-broth at indicated temperatures. At the indicated times, Klett unit of the culture was measured and a portion of the culture was spread on L-broth plate to count viable cell number at 30°C. (o——o): Klett unit of N138recBts1, (•——•): Klett unit of N138recBts1(P2), (Δ——Δ): Viable cell number of N138recBts1, (▲——▲): Viable cell number of N138recBts1(P2).

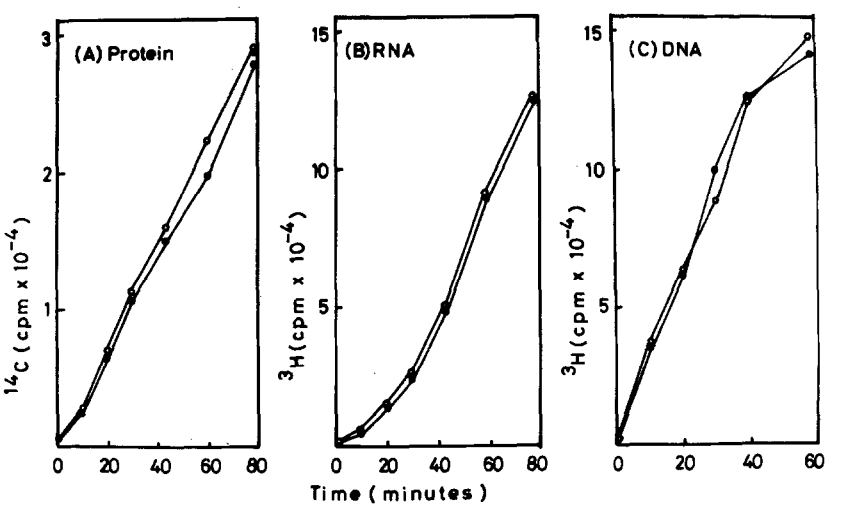

Fig. 2. Macromolecule synthesis in N138recBts1 and its P2 lysogen. Bacteria were grown at 30°C as described in MATERIALS AND METHODS. After addition of [^{14}C] amino acid mixture, [^{3}H]uracil or [^{3}H]thymidine, bacteria were further grown at 42°C. At the indicated time, a portion of culture was taken to measure radioactivity which is insoluble in 5% of trichloroacetic acid. (A): Incorporation of [^{14}C] amino acid mixture, (B): Incorporation of [^{3}H]uracil. (C): Incorporation of [^{3}H]thymidine (o——o): N138recBts1, (•——•): N138recBts1(P2).

Table 1. Plating efficiency of λ phages on recBts(P2) bacteria [a]

Phage	N138recBts		N138recBts(P2)	
	30°C	40°C	30°C	40°C
λcI857reverse (10)	0.92	1.0	0.90	1.0
λcI857bio10 (11)	0.85	1.0	0.21[b]	$<5 \times 10^{-4}$
λcI857bio1 (11)	0.81	1.0	0.24[b]	$<10^{-3}$

a) The plating efficiency of each phage on N138recBts at 40°C was assumed to be 1.0

b) Tiny plaques.

One may argue that the recB$^-$ phenotype introduced by the temperature-sensitive mutation is not sufficient for the P2 old$^+$ gene product to exhibit its effect. To investigate this point, we have tested whether the inactivation of the temperature-sensitive RecBC DNase has an inhibitory effect on the growth of λ in the presence of prophage P2, as the inactivation of the RecBC DNase by the gam protein of λ does. As shown in Table 1, λbio10 and λbio1 which have most of the λ recombination region including the gam gene (11) deleted and are insensitive to the P2 interference (2-4) could not grow on N138recBts(P2) at 42°C. Burst size of λbio10 on N138recBts(P2) and N138recBts was 4 and 40 at 42°C, respectively. Since these phages were able to grow on N138recBts(P2) at 30°C, the failure of these phages to grow on N138recBts(P2) at 42°C was attributed to the inactivation of the RecBC DNase by the temperature-sensitive mutation together with the contributory effects of prophage P2. Thus, the inactivation of the temperature-sensitive RecBC DNase at 42°C is sufficient for P2 old$^+$ gene product to inhibit the growth of λ, as well as in the case of inactivation of the RecBC DNase by the gam protein of λ.

From these results, we concluded that the P2 old$^+$ gene product itself has no killing effect on recB$^-$ hosts and that other phage genes which are expressed in the lytic response are involved in the host-killing process by

the P2 *old*$^+$ gene product. What genes are involved? It has been shown that essential genes of λ, O and P, are involved in the killing of P2 lysogen by phage λ (14). In the case of the killing of *recB*$^-$ hosts by phage P2, it is likely that essential functions of P2 similar to those of lambda O and P are involved since selection for P2 lysogens of *recB*$^-$ bacteria yielded only phage mutated in the *old* gene (1).

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