Short Communication

ROLE OF *gam* PROTEIN AND *RecBC* DNase IN THE GROWTH OF BACTERIOPHAGE λ ON *polA*⁻ HOSTS

SHOJI SHINOMIYA AND YOSHIYUKI SAKAKI*,¹

The Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113

*The Mitsubishi-Kasei Institute of Life Sciences,

11 Minamiooya, Machida-shi, Tokyo 194, Japan

(Received July 26, 1978)

Bacteriophage λ carrying a mutation in *gam* and/or *red* gene cannot multiply in *polA*⁻ hosts (1). SKALKA (2) found that in *polA*⁻ hosts, λ *red*⁻ or *gam*⁻ (or *red*⁻*gam*⁻) cannot produce concatemeric λ DNA which is a precursor of mature λ DNA, and concluded that the growth defect of λ *red*⁻ or *gam*⁻ on *polA*⁻ host is due to the inefficient production of concatemer DNA. Since the production of concatemeric λ DNA through rolling circular replication is sensitive to the host *RecBC* DNase (3) and the *gam* protein of λ is identified as an inhibitor of the *RecBC* DNase (4, 5), SKALKA (2) thought that the *gam* protein is required to protect concatemer DNA production from *RecBC* DNase in *polA*⁻ hosts. This inhibitory role of the *gam* protein is plausible and reasonable, but there remain other possibilities. For example, *gam* and *red* gene may work co-operatively as components of phage-mediated recombination function (1). The *gam* protein might be required in *polA*⁻ hosts as a component of phage recombination function. In order to clarify the role of the *gam* protein in *polA*⁻ hosts, we tested the growth of λ mutant phages on *polA*⁻ hosts in the presence or absence of *RecBC* DNase activity. If the *gam* protein is required as an inhibitor of *RecBC* DNase in *polA*⁻ hosts, λ *gam*⁻ will grow normally on *polA*⁻ hosts in the absence of *RecBC* DNase activity. In this communication, we will show that the role of the *gam* protein in *polA*⁻ hosts is mainly the inhibition of *RecBC* DNase, and also suggest that the impaired temperature-sensitive *RecBC* DNase can help the growth of λ *red*⁻*gam*⁻ on *polA*⁻ hosts.

Since the *polA*⁻*recB*⁻ double mutant is inviable (6), we employed *polA*⁻ hosts carrying a temperature-sensitive *recB* mutation, *recBts1* (7), to make *polA*⁻*recB*⁻

¹ Present address: Department of Biochemistry, School of Medicine, Kyushu University, Fukuoka 812, Japan.

conditions. This temperature-sensitive mutation enables us to make cells *recB*⁺ at 30° but phenotypically *recB*⁻ at 40° (7). As *polA* mutation, we employed two mutants, *polA1* (8) and *polA107* (9), which destroy the DNA polymerase activity and 5'→3' exonuclease activity of DNA polymerase I, respectively. These *polA*⁻*recBts* mutants were inviable at 40°.

We tested the production of progeny phages by λ mutant phages on these bacteria at 30° (*polA*⁻*recB*⁺) and 40° (phenotypically *polA*⁻*recB*⁻). Experimental results are summarized in Table 1. As shown in Table 1, λ *gam*⁻ phage as well as wild type λ can grow well on *polA*⁻ hosts when the *RecBC* DNase was inactivated by temperature-sensitive mutation. (The moderate growth of λ *gam*⁻ on F1022 (*polA1 recBts1*) at 30° is probably due to the low *RecBC* DNase activity in this strain even at 30°.) These results indicate that the major role of the *gam* protein in *polA*⁻ hosts is the inhibition of *RecBC* DNase. On the other hand, λ *red*⁻ phages (λ *red*⁻ and λ *red*⁻*gam*⁻) cannot grow well on *polA*⁻ hosts regardless of the presence or absence of *RecBC* DNase activity, indicating that the *red* function is almost essential for the growth of λ on *polA*⁻ hosts. These results provide strong support for Skalka's postulation.

Curiousness of our results is the moderate growth of λ *red*⁻*gam*⁻ on *polA*⁻*recBts* hosts (F1022 and SSY101) at high temperatures (Table 1). The amount of progeny phages produced under these conditions is sufficient to make plaques on a plate, because λ *red*⁻*gam*⁻ does plate on F1022 (*polA1 recBts1*) at 30°, at which

Table 1. Growth of λ lacking *red* and/or *gam* on *polA*⁻ and *polA*⁻*recBts* hosts.^a

Phages ^e	Bacteria							
	P3478 (<i>polA1</i>)		F1022 (<i>polA1 recBtsI</i>) ^b		KMBL1789 (<i>polA107</i>) ^c		SSY101 (<i>polA107 recBtsI</i>) ^d	
	Temperature (°)							
	30	40	30	40	29	39	29	39
λ^+	1.0 (168)	1.0 (105)	1.0 (91)	1.0 (22)	1.0 (16)	1.0 (100)	1.0 (7.2)	1.0 (76)
λred_{814}	0.06	0.08	0.1	0.09	0.02	0.03	0.03	0.02
λgam_{210}	0.1	0.09	0.36	0.76	<0.01	0.03	<0.01	0.48
$\lambda red_{814} gam_{210}$	0.02	0.02	0.18	0.27	0.02	0.09	0.01	0.15

^a Phages were infected in 10 mM Tris (pH 7.5) and 10 mM MgCl₂ at 30° for 10 min at a multiplicity of 0.1. Phages were grown for 120 min at the indicated temperatures in Tryptone broth (1% Difco Bactotryptone and 0.5% NaCl) supplemented with 1% Difco yeast extract. The values are an mean of results from two experiments for P3478 and F1022 and three experiments for KMBL1789 and SSY101.

^b A gift from Dr. M. SEKIGUCHI. (12)

^c A gift from Dr. B. W. GLICKMAN. (9)

^d The *recBts1* mutation was transduced from N138 (7) to KMBL1789 by PI transduction.

^e *red*₈₁₄ is a mutation in the structural gene for λ -exonuclease. *gam*₂₁₀ is a nonsense mutation in the *gam* gene. All phages carried i⁴³⁴*cI* mutation to prevent the phage from lysogenizing.

Table 2. Burst size of λ *red*⁻*gam*⁻ on *polA*⁻*recB*⁻ host.^a

Phages	Bacterium	
	ST417 (<i>polA</i> _{ts12} <i>recB</i> ₂₁) ^b	
	Temperature (°)	
	30	40
λ ⁺	1.0 (64)	1.0 (123)
λ <i>gam</i> ₂₁₀	0.34	0.68
λ <i>red</i> ₁₁₄ <i>gam</i> ₂₁₀	0.11	0.02

^a Experimental condition and symbol are the same as in Table 1.^b A gift from Dr. S. Takahashi.

temperature *RecBC* DNase activity is somewhat low in F1022. This moderate growth of λ *red*⁻*gam*⁻ cannot be explained by the known functions of *red*, *gam*, *polA*, and *recB*. Two explanations may be possible: (a) The *gam* protein has an unknown inhibitory effect on the growth of λ in *polA*⁻ host or (b) the impaired temperature-sensitive *RecBC* DNase somehow supports the growth of λ on *polA*⁻ hosts. In order to clarify which possibility is plausible, we tested the production of progeny phages by λ *red*⁻*gam*⁻ on *recB*⁻ host carrying a temperature-sensitive *polA* mutation, *polA*_{ts12} (6). λ *red*⁻*gam*⁻ will show a moderate growth at high temperatures if possibility (a) is true, but will not grow if possibility (b) is correct. As shown in Table 2, λ *red*⁻*gam*⁻ could not grow at all on *polA*_{ts12} *recB*⁻ at 40°. These results suggest that the possibility (b) is more plausible. *RecBC* DNase has four catalytic activities; exonuclease activity for double-stranded and single stranded DNA, endonuclease activity for single-stranded DNA, and DNA-dependent ATPase activity (10). It has been reported that the temperature-sensitive *RecBC* DNase employed in this work loses only an exonuclease activity for double-stranded DNA among these four activities at high temperatures (11). So far, there has not been any evidence indicating that the impaired *RecBC* DNase has some biological function, except our case. Further study is, however, required to clarify the role of the impaired *RecBC* DNase in the growth of λ .

ENQUIST and SKALKA (3) have shown that the concatemeric λ DNA is produced through "replicative," "*red*-dependent," or "*rec*-dependent" pathway. According to this pathway concept, our results can be summarized as follows: In the absence of DNA polymerase I, only *red*-dependent pathway can work and a crucial step of this *red*-dependent pathway is sensitive to host *RecBC* DNase. The *gam* protein is required to protect this *red*-dependent pathway from *RecBC* DNase. In addition to three known pathways, there exists a minor pathway where the impaired temperature-sensitive *RecBC* DNase plays a certain role.

We thank Dr. T. Oshima of the Mitsubishi-Kasei Institute of Life Sciences, and Dr. B. Maruo of the Institute of Applied Microbiology, University of Tokyo, for their encouragement and support, and to Drs. M. Sekiguchi, B. W. Glickman, and S. Takahashi for the donation of bacterial strains.

REFERENCES

- 1) J. ZISSLER, E. SIGNER, and F. SCHAEFER, *In* The Bacteriophage Lambda, ed. by A. D. HERSHEY, Cold Spring Harbor Laboratory, New York (1971), p. 455.
- 2) A. SKALKA, *In* Mechanism in Recombination, ed. by R. F. GRELL, Plenum Press, New York (1974) p. 421.
- 3) L. ENQUIST and A. SKALKA, *J. Mol. Biol.*, **75**, 185 (1973).
- 4) R. UNGER and A. J. CLARK, *J. Mol. Biol.*, **70**, 539 (1972).
- 5) Y. SAKAKI, A. KARU, S. LINN, and H. ECHOLS, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2215 (1973).
- 6) M. MONK and J. KINROSS, *J. Bacteriol.*, **109**, 971 (1972).
- 7) J. TOMIZAWA and H. OGAWA, *Nature New Biol.* (London), **239**, 14 (1972).
- 8) P. De Lucia and J. Cairns, *Nature* (London), **224**, 1164 (1969).
- 9) B. W. GLICKMAN, C. A. van SLUIS, H. L. HEIJNEKER, and A. RÖRSCH, *Mol. Gen. Genet.*, **124**, 69 (1972).
- 10) P. GOLDMARK and S. LINN, *J. Biol. Chem.*, **247**, 1847 (1972).
- 11) S. R. KUSHNER, *J. Bacteriol.*, **120**, 1219 (1974).
- 12) M. KATSUKI and M. SEKIGUCHI, *Biachim. Biophys. Acta*, **383**, 188 (1975).