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Pseudo T-even bacteriophage RB49 displays rapid lysis phenotype

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Throughout the long history of the bacteriophage T4 research, the phenomenon of lysis inhibition (LIN) became an exclusive point of interest of T4 physiology. An ability to control the timing of cell lysis in response to the availability of bacterial hosts in the environment is considered to contribute significantly to the prevalence of T-even phages in nature. In this study, we investigated lysis inhibition in the significantly diverged pseudo T-even bacteriophage RB49. Although the phage RB49 possesses a highly diverged homologue of the T4 major lysis inhibition gene *rI*, the *Escherichia coli* B^E and K-12 cells infected with RB49 do not display lysis inhibition. In addition, we demonstrated that secondary infection by RB49 phage induces the lysis inhibition of T4-infected cells. The evidence suggests that phylogenetically distant T4-type phages have elements that constitute a signal of the LIN induction.

Key words: bacteriophage RB49; rapid lysis, gene *rI*; lysis inhibition

INTRODUCTION

The exceptional feature of T4 and the closely related T-even bacteriophages is lysis inhibition. This is a unique strategy to regulate the yield of virus particles in response to the availability of susceptible host cells in the environment [1]. Normally, T4-infected cells lyse 20–30 min after infection. If the infected cell undergoes a secondary infection 3 min after the primary infection, lysis is inhibited and the infection cycle extends with a consequent increase in the burst size. Rapid-lysis (r) mutants fail to delay lysis in response to secondary infection. This results in large sharp-edged plaques that are easily distinguishable among small rough-edged plaques formed on agar lawns by a wild-type phage [2, 3]. Recently, it has been established that interaction between the holin T and antiholin encoded by gene *rI* plays the major role in the mechanism of lysis inhibition [4, 5]. However, the nature of the signal activating lysis inhibition remains obscure. Lysis inhibition of T4-infected cells can be triggered by other T-even phages but not by phage ghosts whose heads have been broken by osmotic shock [6]. Based on this finding, it has been proposed that LIN signal is constituted by the elements missing from phage ghosts: DNA, the internal proteins IPI, IPII or IPIII, and the internal peptides generated during cleavage of the head proteins [1]. Secondary infection with unrelated phages such as T5 and P2 cannot induce LIN in T4-infected cells [6].

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The apparent competitive advantage provided by lysis inhibition makes it seem likely that LIN is widely conserved among T4-related phages. The gene *rI* that encodes the homologue of the basic component of the lysis inhibition mechanism could be found in the genomes of all T-even phages sequenced to date (see http://phage.bioc.tulane.edu). Moreover, the gene *rI* sequence similarity is conserved for a more distantly related T4-type phages that belong to the pseudo T-even subgroup. Recently, we have determined the gene *rI* sequence from the pseudo T-even bacteriophage RB49 [7]. The primary structure predicted for the protein encoded by this gene exhibits only a 20% identity to the gprI of T4 at the amino acid sequence level. Together with the highly diverged protein sequence, we found that the expression pattern of the gene rI of RB49 interferes with the gprI function in lysis inhibition because of the stable RNA secondary structure in the translation initiation region [7].

In this study, we present lysis kinetics produced by phage RB49. The obtained results show that under the standard laboratory conditions RB49 displays a rapid lysis phenotype.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *Escherichia coli* strain B^E (*sup*^{0}) was a gift from Dr. Lindsay W. Black. *E. coli* strain K-12 (*sup*^{0}) was obtained from Dr. Victor Krylov.

Bacteriophages T4D wild-type and RB49 were kindly supplied by Dr. William B. Wood. T4 *rI* mutant *r*48 was kindly

provided by Dr. Karin Carlson. The T4 gene *26* double mutant *26am133-26am165* was constructed in our laboratory.

Lysis inhibition assays. *E. coli* cells were grown overnight in a standard Luria–Bertani (LB) broth. 5 ml LB were inoculated with 0.05 ml of an overnight culture and the diluted cells were grown at 37 °C with rigorous aeration (200 rpm) to an $A_{600} = 0.8$. Based on an assumed cell titer of 2×10^8 cells/ml at $A_{600} = 0.8$, phages were added to the flasks to give a multiplicity of infection (MOI) of 10. After 10 min, phage at MOIs of 10 was added to the culture to achieve secondary infection. Starting at 0 min (time of initial infection), absorbance at 600 nm was measured at appointed times with a spectrophotometer (Eppendorf Biophotometer). All values were normalized to the value at 0 min.

RESULTS AND DISCUSSION

Lysis profile of RB49

It has been previously noticed that the titers of RB49 stocks produced on *E. coli* strain B^E are typically by a factor of 10 lower than those produced by T4 [8]. Appealing to the role that T4 early promoters play in the rapid takeover of the host transcription machinery [9, 10], it was suggested that low titers of RB49 stocks are conditioned by the absence of discrimination between the *E. coli*-like early promoters of RB49 and host promoters early in infection [8]. However, we observed that the plaques of RB49 produced on B^E cells resemble those of *r*-type of T4 (Fig. 1). Therefore, it is reasonable to suspect that the *r*-plaque morphology and the considerably lower titers of phage stocks may actually reflect the absence of lysis inhibition in RB49-infected cells.

In order to evaluate the potential of RB49 to undergo lysis inhibition, we monitored the lysis profiles of RB49-infected *E. coli* B^E cells. As a control we used B^E cells infected by T4D wild-type and the gene *rI* mutant *r*48 that represented lysis profiles with and without LIN, respectively. Lysis profiles of RB49-infected cells presented in Fig. 2 show that RB49 displayed almost the same kinetics of cell lysis in both cases, i. e. with and without secondary infection. Except for the earlier lysis start, the overall lysis pattern for the RB49 was very similar to that of T4 *r*48 mutant. Considering the fact that some of T4 *r* mutants show *r* phenotype on some host cells but not on the others [11], we also examined lysis kinetics of *E. coli* strain K-12 infected with RB49 and observed very similar results to that gained with B^E cells (not shown).

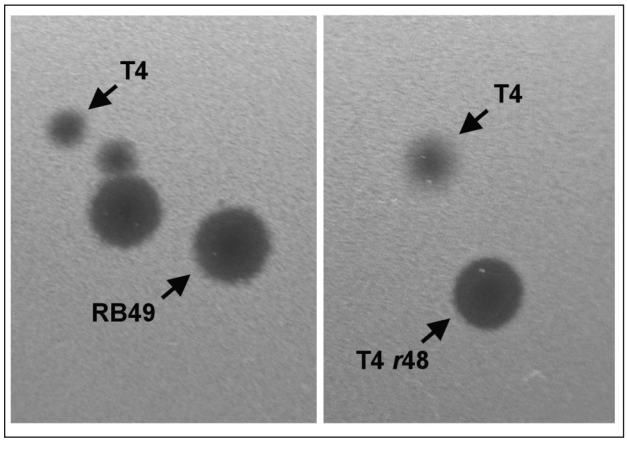

Fig. 1. Phage plaques formed on agar lawn of *E. coli* B^E by T4D wild-type, RB49 and T4D mutant *r*48

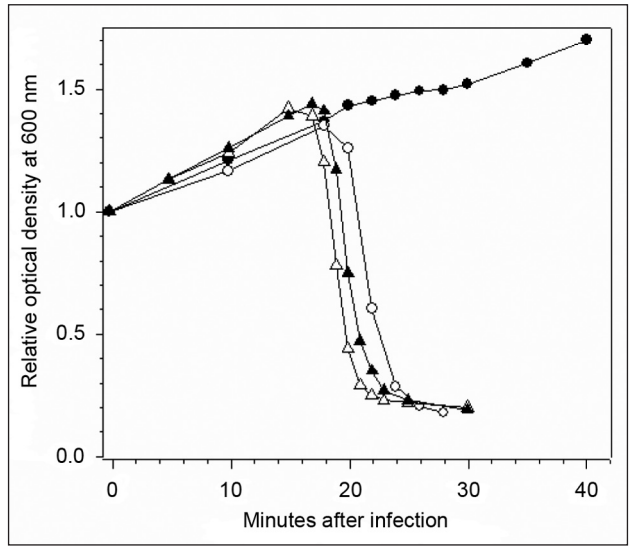

Fig. 2. Lysis profiles of the pseudo T-even phage RB49 without (Δ) and with (▲) secondary infection, compared with the lysis profiles of T4D (●) and T4D mutant *r*48 (○) with secondary infection. *E. coli* B^E cells were grown in LB broth at 37 °C to a concentration of 2×10^8 and infected with phages at MOI of 10. Secondary infection at MOI of 10 was carried out 10 min later with the same phage used for initial infection

The obtained results clearly indicate the inability of RB49 to prolong its infection cycle in response to secondary infection, in spite of the fact that RB49 owns the gene *rI* that encodes the basic component of the lysis inhibition mechanism. Sequencing of the RB49 genomic region between the genes *31* and *30* revealed no gene homologous to the gene *rIII* of T4 (EMBL/GenBank accession No. AJ550428). Therefore, the absence of gene *rIII* in the genome of RB49 could be the simplest explanation for the rapid lysis phenotype of RB49. The product of gene *rIII* is required for LIN manifestation in the T4-infected *E. coli* strains B^E or K-12, albeit it is not clear to which extent the gprIII is implicated in this process [1, 4]. Based on these observations, one could assume that while the RB49 has the functional gprI, it fails to display lysis inhibition due to absence of gprIII. Substantial alterations of the expressional control of RB49 gene *rI* could be another possible reason for the absence of LIN in RB49 [7]. Considering the observed concordance between the mode of expression and the function of T4 gene *rI* [1, 12], the shift of RB49 gene *rI* into the class of late genes that are transcribed into the early and late transcripts, though translated from the late ones because of inhibitory RNA stem-loop structures, may result in the loss of lysis inhibition function. However, other explanations including another function for gene *rI* of RB49 should not be ruled out.

Secondary infection of the T4-infected cells by RB49 phage

Because the most reliable candidates for the LIN signal are the phage DNA or the internal proteins [1], there is a possibility that the elements constituting the LIN signal of more distant T4-related phages have diverged from those of T4. Therefore, it is reasonable to expect that in the case of secondary infection of

the T4-infected cells by the more distant T4-related phage we will observe a different LIN induction. To test this presumption, we examined the lysis kinetics of cells primarily infected by T4 and secondarily infected by RB49 phage. For the initial infection we used a T4 base-plate gene *26* double *amber* mutant (*26am133-26am165*) and carried out the experiment in the B^E (*sup*^{0}) host. These conditions eliminate the possibility of secondary infection because the progenies of the mutant phage are unable to adsorb. This permits to observe lysis of T4-infected cells without LIN. Secondary infection with the T4D wild-type phage induces a usual LIN state in cells primarily infected by the T4 *26am133-26am165* mutant (Fig. 3). In case of secondary infection by RB49, a decrease in the time of the normal lysis and the successive stabilization of the culture turbidity was observed.

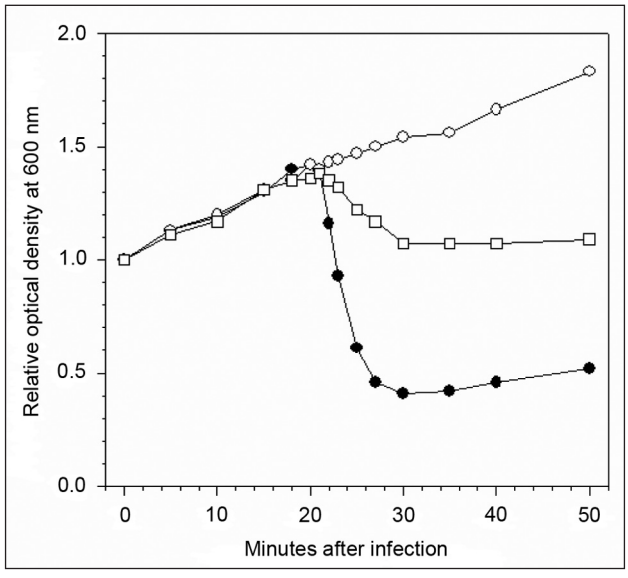

Fig. 3. Lysis profiles of the T4 *26*$^-$ mutant superinfected with T4D wild-type (○), pseudo T-even phage RB49 (□), and without secondary infection (●). *E. coli* B^E cells were infected with T4 *26*$^-$ at a MOI of 5 and 10 min later superinfected with appropriate phage at a MOI of 20

The results reported here suggest that T4-type phages have elements that constitute the LIN signal. However, induction of lysis inhibition by RB49 secondary infection is apparently less effective. It should be noted that no genes were found to encode the internal proteins IPI, IPII and IPIII in the genome of pseudo T-even phage RB49 (13). Therefore, it is likely that the absence of internal proteins in the head of the superinfecting phage RB49 could be the reason for an incomplete lysis inhibition in T4-infected cells. On the other hand, partial induction of LIN may signify the weaker interactions between the yet unknown LIN signal of RB49 and the T4 signal receptor.

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PSEUDO T LYGINIAM BAKTERIOFAGUI RB49 BŪDINGAS GREITOS LIZĖS FENOTIPAS

Santrauka

Išskirtinis bakteriofago T4 ir jam gimininguų T-lyginių fagų bruožas yra lizės inhibicijos fenomenas. Tai unikali strategija, kurią T lyginiai fagai naudoja maksimaliam savo skaičiaus padidinimui, kai aplinkoje trūksta infekcijai tinkamų bakterinių ląstelių. Manoma, kad sugebėjimas reguliuoti infekcijos ciklo trukmę ir kartu išsivystančių virionų kiekį turi didelę reikšmę plačiam šių bakteriofagų paplitimui gamtoje. Šiame darbe buvo tiriamas RB49bakteriofagas, kuris priklauso filogenetiškai tolimesnių pseudo T lyginių fagų grupei. Mes nustatėme, kad RB49 bakteriofagu infekuotose ląstelėse nevyksta lizės inhibicija, nors šio fago genome yra pagrindinis lizės inhibicijos genas *rI*. Taip pat buvo nustatyta, kad RB49 fagu infekuojant prieš tai T4 fagu infekuotas ląsteles gali būti indukuota lizės inhibicija. Šie duomenys rodo, kad RB49 fagas turi lizės inhibiciją įjungiantį signalą.