Analysis of the Phenotypic Effect of the ts47 Mutation of the Pseudomonas aeruginosa Transposable Phage D3112 on the Expression of Late Phage Genes

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SHORT COMMUNICATIONS

Analysis of the Phenotypic Effect of the *ts47* Mutation of the *Pseudomonas aeruginosa* Transposable Phage D3112 on the Expression of Late Phage Genes

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Abstract—At nonpermissive temperature (42°C) the *ts47* mutation causes substantial abnormalities in the late phase of the phage intracellular development. In these conditions DNA of the D3112 phage is detected both in a free form and integrated into bacterial chromosome. The transcription kinetics in the *ts47* mutant at 42°C was indistinguishable from that typical to other early gene mutants (*A, B,* and *C*): specifically, the preservation of the first transcription peak along with low activity of late transcription were observed. Similarly to the *C* gene, the *ts47* mutation-carrying locus is involved in regulating the transcription of the D3112 transposable phage late genes. It is suggested that the mechanism underlying the action of the *ts47* mutation differs from that of the *C* gene product. One of the possible explanations is based on the fact that the product of the *ts47* locus affects the activity of cellular RNA polymerase via providing more effective recognition of the phage promoters by the RNA polymerase modified with the phage protein C.

INTRODUCTION

Intracellular development of the *Pseudomonas aeruginosa* transposable phage (TP) D3112 starts with DNA replication, which is followed by the synthesis of the proteins essential for the morphogenesis of the phage particles. This synthesis depends on the activity of the *C* gene, whose product is capable of anti-terminating activity. In this work, we show that TP D3112 contains an additional genome region (marked so far by a single *ts47* mutation, and defined as the *ts47* locus) which along with the *C* gene affects the phage protein synthesis. The lack of this locus results in the decreased levels of the late gene transcription.

Bacterial strains. We used the *Pseudomonas aeruginosa* PAO1 strain, kindly provided by B. W. Holloway (Australia).

Bacteriophages of P. aeruginosa. The phages used were TP D3112 *cts15* (the *cts15* mutation is located in the *cI* gene, which is capable of controlling the repressor and makes the prophage thermoinducible at 42°C; TP D3112 *cts15 ts104*, which in addition to the *cts15* mutation carries the *ts* mutation in the *C* gene, the positive regulator of the D3112 late genes transcription; TP D3112 *cts15 ts47*, which in addition to the *cts15* mutation carries the *ts47* mutation [1]; and E79 virulent phage, kindly provided by B. W. Holloway (Australia).

The following derivatives of the RP4::D3112 hybrid plasmids carrying defective prophages (in brackets are physical coordinates on the plasmid ends in the prophage part of the genome) were used: pDT18 (0–1.8); pDT36 (0–14.5); and pKT31 (0–22.0) [1].

The methods of working with bacteriophages are described in [2].

Media. We used Hottingen agar and broth as complete nutrient media. Minimal media used were M9 agar and broth [3].

Experiments on preparation of the ^{3}H-RNA samples were carried out using M9 medium [3].

Buffer solutions are described in [3] and [4].

Artificial lysis of the *P. aeruginosa* cells was carried out by use of lysozyme (final concentration of 2 mg/ml) or the E79 virulent phage excess (infection multiplicity = 100).

Induction of lysogens with thermoinducible prophages was carried out as follows: night culture of lysogenic bacteria was diluted from 50 to 100 times and grown up to a concentration of $(3–5) \times 10^{8}$ cells/ml at 30°C with aeration and agitation at 100 rpm using the G76 (New Brunswick, United States) shaker. Next, the samples were incubated upon active aeration at 42°C for 90–120 min.

Isolation of total cellular DNA is described in [5].

Transfer of the DNA samples onto nitrocellulose filters, DNA–DNA hybridization, and preparation of the ^{32}P-DNA samples were conducted as in [4]. Obtaining preparations of ^{3}H-labeled RNA and RNA–DNA hybridization were carried out according to [6] and [7].

The ts47 mutation does not prevent the lysis of bacterial cells after thermoinduction of the PAO1 (D3112 *cts15 ts47) lysogen.* The D3112*cts15 ts47* mutant was isolated by use of the method providing selection of mutant phages by the early genes, i.e., the mutants whose lysogenic derivatives were not subjected to lysis during phage development after thermoinduction were isolated [1].

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Titers of viable phage after the induction of different lysogens

Incubation time, min	Phage titers				
	D3112*cts15*	D3112*cts15 ts104*	D3112*cts15 ts47* after the treatment with		
			$CHCl_3$	lysozyme	E79 phage
0	3×10^7	8×10^6	1×10^7	2×10^8	2×10^8
30	6×10^9	9×10^6	7×10^7	2×10^8	3×10^8
60	1×10^{11}	2×10^7	2×10^8	3×10^8	4×10^8
90	2×10^{11}	2×10^7	1×10^9	2×10^9	3×10^9

The *ts47* mutation was mapped to the region between the 13.5 and 21.0 kb of the phage physical map [1]. The D3112*cts15 ts47* phage differs from its wild-type counterpart by the NC (negative colony) phenotype on the PAO1 lawn under permissive temperature (it forms turbid NC).

The tests for the *ts47* complementation carried out by use of drop analysis during combined infection of the cells by the *ts* mutants for other early genes (*A, B*, and *C*) were positive in all cases. These data permit considering the *ts* mutation as a novel gene. This statement, however, is not conclusive, since only single specimen of the mutant with the given phenotype and location has been found so far. For these reasons, we shall further define this hypothetical gene as the *ts47* locus. First, we shall consider that this locus controls the lysis of bacterial cells at the end of the phage vegetative growth. Testing of this proposal (measurement of the turbidity of the b-induced lysogens incubated at 42°C) showed, that in these conditions the lysis of the PAO1 (D3112*cts15 ts47*) bacteria actually took place (suspension turbidity decreased and its viscosity increased). This lysis, however, occurred somewhat later than that of the PAO1 (D3112*cts15*) cells. This delay probably led to selection of the *ts47* mutant in the experiment designed for the selection of nonlytic *ts* mutants.

The data on the effect of the *ts47* mutation on the production of the D3112 phage under nonpermissive temperature are presented in the table.

Our data show that after induction of the PAO1 (D3112*cts15 ts47*) lysogen and spontaneous lysis, the phage titer is 1 to 5×10^2 times diminished compared to the control (the D3112*cts15* phage upon initially equal titer of lysogenic bacteria, and the free phage, spontaneously induced in suspension prior to thermoinduction). At the same time, the titer of the D3112*cts15 ts47* phage was higher than that observed for the D3112*cts15* (data not shown) and the *C* early gene mutation-carrying D3112*cts15 ts104* phage.

This finding suggests incomplete suppression of the D3112*cts15 ts47* development at 42°C. Experiments on artificial cell lysis by use of lysozyme, or the E79 phage gained similar results.

Genetic analysis of the D3112cts15 ts47 mutant. The fact of the absence of the revertants capable of the growth at 42°C upon plating of the large amounts of the D3112*cts ts47* phage (about 10^{11} phage particles) in nonpermissive conditions, has led to the proposal that genome of the mutant phage carries not a single but two or more *ts* mutations. To test this proposal, the crosses between the D3112*cts15 ts47* and a number of defective prophages integrated into hybrid plasmids were carried out. The corresponding plasmids were introduced in PAO1 (D3112*cts15 ts47*) cells via conjugation. As a result, the following dilysogens were obtained: PAO1 (D3112*cts15 ts47*, pDT18), PAO1 (D3112*cts15 ts47*, pKT31), PAO1 (D3112*cts15 ts47*, pDT36), and PAO1 (D3112*cts15 ts47*, pKT31). From the phage progeny of the three dilysogens carrying the pDT18, pKT31, and pDT36 plasmids, hybrid phages with similar phenotypes forming transparent NC and capable of the growth at 42°C were selected. It is noteworthy that these recombinants were not found among the progeny of the cross between the D3112*cts15 ts47* phage and the prophage within the pKT31 plasmid. This finding confirms the localization of the *ts47* mutation on the genetic map of the D3112 [1]. Lysogens of the ten recombinant phages selected in each successful cross (a total of 30 lysogens), were selected and subjected to thermoinduction at 42°C. All the phages selected exhibited the phenotype typical to the D3112*cts15* (lysis of the lysogens occurred after the induction and simultaneously with the lysis of the lysogen carrying the D3112*cts* prophage; the phage yields in both cases were also similar).

Since no novel recombinant types were observed, the low frequency of the *ts47* reversion can be explained by the close linkage between two hypothetical independent mutations or by the property of the given mutation.

Analysis of the DNA replication in the TP D3112 carrying the ts47 mutation. To determine the stage of the phage growth, at which the action of the hypothetical *ts47* mutation-carrying gene product is manifested, replication and transposition of the TP DNA after thermoinduction of the PAO1 (D3*cts15 ts47*) lysogen was carried out. We showed that the processes of replica-

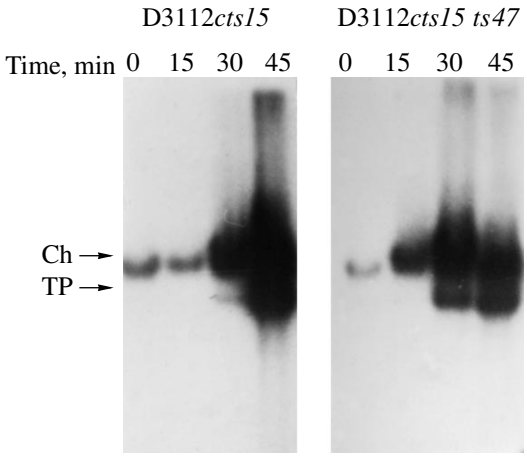

Fig. 1. Analysis of the effect of the *ts47* mutation on replication–transposition of the D3112 phage DNA. Induction of the prophages in lysogenic bacteria *P. aeruginosa* PAO1 (D3112*cts15 ts47*) and PAO1 (D3112*cts15*) (control) and incubation of bacterial cells were carried out at 42°C. Samples for the DNA isolation were collected 0, 15, 30, and 45 min after the induction. Total DNA samples isolated from the two lysogens were separated through electrophoresis on 4% agarose gels and subjected to the DNA–DNA Southern hybridization with the D3112 ^{32}P-DNA probes. Bacterial chromosomal DNA (Ch) and mature D3112 phage DNA (PT) samples are indicated by the arrows.

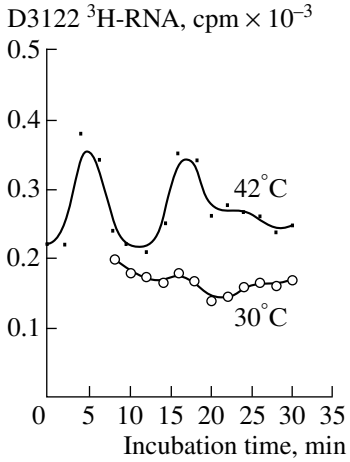

Fig. 2. Analysis of the effect of the *ts47* mutation on transcription of the D3112 phage. The curves show synthesis of the D3112*cts15 ts47* ^{3}H-RNA at two different temperatures.

tion–transposition in the *ts47* mutant were not disturbed: DNA replication, transposition and maturation patterns were similar to those observed in the wild-type phages (Fig. 1). The host cells contained the D3112 phage DNA. Furthermore, this DNA was present in both integrated into the host chromosome and the free forms. The latter result was rather surprising and could not be interpreted unambiguously. First, it is possible that excision of the phage DNA from bacterial chromosome in the course of the TPD3112 development some times can occur independently from the packing processes. Alternatively, it cannot be excluded that in the presence of *ts47*, DNA is packed in some unstable structures, which further are subjected to destruction. Indeed, excision of the phage DNA in the *ts47* mutant (measured as the amount of DNA indistinguishable from that of the mature phage) is not disturbed, while the value of the mature phage yield is lower by the two orders of magnitude.

Analysis of the genome transcription in TP D3112 carrying the ts47 mutation. Examination of the DNA transcription kinetics in phage D3112*cts ts47* at 42°C (after thermoinduction of the lysogen by this prophage) showed that it was indistinguishable from that typical to other phages with early gene mutations (*A, B*, and *C*). Specifically, the first transcription peak corresponding to the early RNAs was preserved and low activity of the late transcription (Fig. 2) corresponding to the transcription of the late TP D3112 genes [8] involved in the control of the morphogenesis of the phage particle [9] as observed.

Experiments on the change of the incubation temperature of the PAO1 (D3112*cts ts47*) cells after the induction at 42°C followed by the transfer of the cells to 30°C showed that after incubation at 30°C the absolute amount of the phage RNA did not increase, while effectiveness of the late transcription increased, since the ratio between the phage and the host RNA remained unchanged. Specifically, a temperature decrease from 42 to 30°C resulted in a considerable reduction in the incorporation of the ^{3}H-uracil into cellular RNA (Fig. 2). This did not occur in the control experiments with the D3112*cts15* phage.

Thus, similarly to the *C* gene product, the *ts47* mutation did not affect late transcription. However, since the *ts47* and *ts104* mutations complement, it can be suggested that the *C* gene in the *ts47* mutant is not damaged. Consequently, similarly to the *C* gene, this hypothetical gene is directly involved into the regulating of the transcription of the late TP D3112 genes. The lack of the active product of this gene results in the decreased levels of the transcription of the late genes, which in turn, leads to the decreased production of their proteins, essential for morphogenesis.

In TP D3112 of *P. aeruginosa* a novel locus, marked by the *ts47* mutation, has been characterized. A functional analogue of this locus in the Mu phage genome has not been discovered so far. Mutation in this locus has no influence on the replication–transposition of the phage DNA. The product of this locus along with the gp C, have been found to be essential for the normal level of the late transcription. Contrary to the situation observed in another *C* gene mutant (*ts104*), in the case of the D3112*cts ts47* phage the incubation of the lysogenic cells at 30°C after the induction at 42°C did not lead to the increase of the phage RNA synthesis (Fig. 3). This process, however, was accompanied by an increase of the effectiveness of the phage DNA transcription due to attenuation of the synthesis of the cel-

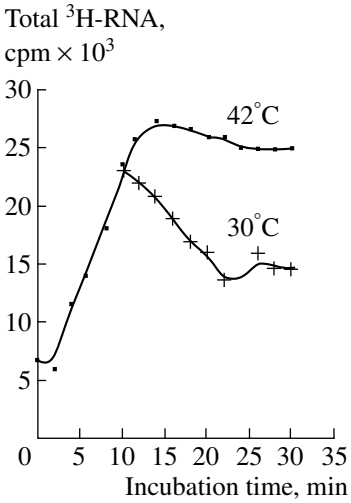

Fig. 3. The effect of the *ts47* mutation on synthesis of the total cellular ^{3}H-RNA. *P. aeruginosa* (D3112*cts15 ts47*) cells were either incubated during the whole experiment at 42°C (upper curve) or induction was carried out at 42°C for 6 min, and incubation was performed at 30°C (lower curve).

lular RNA and maintenance of the constant level of the phage RNA synthesis.

The reasons for the observed in the present study appearance of the mature phage DNA and the lack of its packing into phage particles remain to be determined (see above).

It is suggested that the mechanism underlying the action of the given gene product differs from that of the C protein. One of the possible explanations is based on the fact that on the certain stages of the phage growth the product of the *ts47* locus inhibits the activity of cellular RNA polymerase, providing more effective recognition of the phage promoters by the RNA polymerase modified with the phage protein C.

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