Transcriptional Mapping and Studying the Control of Transcription of the Pseudomonas aeruginosa Transposable Phage D3112

Elena Bidnenko, V. Akhverdyan, V. Krylov

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Transcriptional Mapping and Studying the Control of Transcription of the *Pseudomonas aeruginosa* Transposable Phage D3112

E. M. Bidnenko, V. Z. Akhverdyan, and V. N. Krylov

State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow, 113545 Russia

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Abstract—Regulation of transcription was studied in a wild-type transposable phage (TP) D3112 of *Pseudomonas aeruginosa* and its mutants for different genes. For this purpose, [^{3}H]RNA–DNA hybridization with denatured fragments from various regions of the D3112 genome bound to nitrocellulose filters was used. A transcriptional map of TP D3112 was constructed based on the data obtained. The map comprised six independent transcriptional units corresponding to the modular organization of the phage genome. Only the repressor gene *cI* was transcribed in the lysogenic state. After repressor thermoinactivation, the *cI* transcription ceased, and transcription occurred in the same order as the genes (modules) were located on the D3112 phage genetic map (from left to right): *cip1* (a negative regulator) and early genes *A* and *B* (controlling the replication–transposition of the phage), nonessential genome region, genes *C* and the locus marked with the *ts47* mutation (positive regulators of late gene transcription), the genes of the head morphogenesis, gene *c91* (a positive regulator responsible for the lysogenic state), and the genes of the tail morphogenesis. Similarities between nonhomologous TPs D3112 of *P. aeruginosa* and Mu of *Escherichia coli* with respect to genetic organization and transcription regulation are discussed.

INTRODUCTION

The *Pseudomonas aeruginosa* transposable phage (TP) D3112 and *Escherichia coli* TP Mu has similar genetic organizations [1–5], although they are unrelated and do not display DNA–DNA homology. It was found that repressor genes (*cI*)p; regulatory genes *cip1* in D3112 and *ner* in Mu; and genes *A* and *B*, which are responsible for replication–transposition in both TPs, [6–12], as well as the nonessential genome region, regulators of late gene transcription, and genes controlling morphogenesis of the head and the tail [8, 13, 14] were located similarly in the genomes of phages D3112 and Mu. In addition, genes *cI*, *A*, *B*, and, to a certain extent, *cip1* and *ner* had similar functions in the two TPs.

Further studying functional homology between the genes and gene groups of TPs D3112 and Mu required a more detailed analysis of the roles of different genes involved in the regulation of the phage expression. When studying the transcription of TP D3112 (from *P. aeruginosa*) genome in a homologous host, two transcription waves were observed, which corresponded to the early and late RNA syntheses [15]. Early RNA synthesis was independent of the D3112 replication–transposition, whereas late RNA synthesis required the products of phage genes *C* and the locus containing the *ts47* mutation site [16] (hereinafter, locus *ts47*), as well as effective replication of the phage [15, 16].

In this study, we carried out transcriptional mapping of the D3112 genome. The data obtained allowed us to determine the order in which different genomic regions of the phage are transcribed and to estimate the transcription rate during the lytic development. We suggest a transcriptional map comprising six independent transcriptional units corresponding to the main modules of phage D3112. This map implies that the regulatory patterns of the *P. aeruginosa* TP D3112 and the *E. coli* TP Mu are similar to each other. The roles of the regulatory genes *cI* and *cip1* (repressors of early gene transcription), as well as gene *C* and locus *ts47* (positive regulators of late gene transcription) are discussed.

MATERIALS AND METHODS

Bacterial strains. P. aeruginosa strain PAO1 was obtained from B.W. Holloway (Australia).

Bacteriophages. We used phage D3112*cts15* carrying mutation *ts* in the *cI* gene and *ts* mutants of this phage for essential genes, including *ts17*, *ts71*, *ts104*, and *ts47* [4].

D3112*cts15* phages with polar mutations, whose phenotypes were $CIP^+A^-B^-X^-C^+$ and $CIP^-A^-B^-X^-C^+$, as well as D3112*ts15m* and B39s mutants [4] were used.

The methods used in experiments with bacteriophages are described in [17].

Media. Hottinger's medium (meat digest) was used, in the forms of liquid medium and agar medium, to obtain and titrate the phage. The M9 basic medium [18] was used in experiments where [^{3}H]RNA was obtained.

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The buffer solutions in this study were described in [18, 19].

[^{3}H]RNA was obtained as described in [15, 20]. An overnight culture of PAO1 (D3112*cts15*) or another lysogenic PAO1 strain with an additional mutation in the D3112 phage genome was grown in the basic medium, diluted 1 : 50, and grown additionally at 30°C under intense aeration to obtain a concentration of $(3–5) \times 10^8$ cells/ml. Samples of the culture were incubated at 42°C to induce the prophage. [^{3}H]Uracyl (50 μl/ml of culture) was added at different moments after the induction. After 2 min of incubation with the labeled uracyl, the samples were rapidly cooled on ice, and sodium azide was added to a final concentration of 20 mM. RNA was isolated by the phenol-detergent method and treated by RNase-free DNase (20 μg/ml) at 20°C for 30 min and by proteinase K (50 μg/ml) at 37°C for 1h.

Isolation of phage DNA and isolation of restriction fragments of phage D3112 DNA from the agar gel was conducted as described in [19].

Immobilization of DNA and DNA fragments of phage D3112 on nitrocellulose filters. An equal volume of 0.1 M NaOH was added to the 0.5–1 μg/μl DNA solution. The mixture was incubated at 100°C for 15 min and rapidly cooled to 4°C. The alkali was neutralized with an equimolar amount of 1 N HCl. One volume of the 20 × SSC buffer was then added, and samples of the mixture were immediately applied to nitrocellulose filters (Millipore, United States) with the pore diameter 0.45 μm preliminarily washed in a 6 × SSC buffer. We applied 5–25 μg of DNA per filter. The filters were dried in the air and annealed in vacuum 1t 80°C for 2 h.

[^{3}H]RNA–DNA hybridization. The filters that were used for DNA–RNA hybridization were preliminarily incubated in Denhardt's solution at 65°C for 4h. Afterwards, the filters were placed into the hybridization mixture containing [^{3}H]RNA and the 6 × SSC buffer.

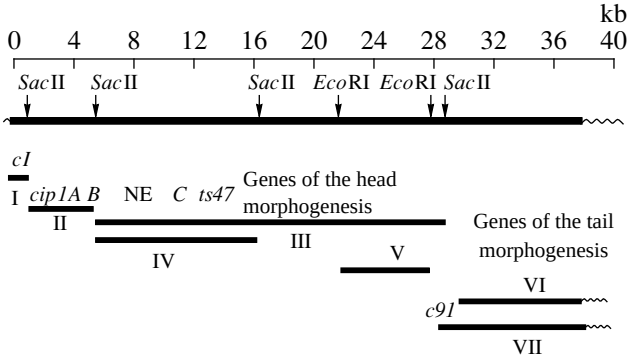

Fig. 1. The physical map of phage D3112 and DNA restriction fragments obtained after treatment with restriction endonucleases *Eco*RI and *Sac*II. The fragments shown in the figure were used in experiments on RNA–DNA hybridization. Phage genes mapped to each genomic region are indicated above the fragments. NE is the nonessential region of the phage D3112 genome.

Hybridization was performed at 65°C for 16–18 h. After hybridization, the filters were washed with the 2 × SSC buffer several times at the same temperature and treated with RNase at a final concentration of 25 μg/ml at 37°C for 30 min. The filters were washed to remove RNase and dried in the air. Radioactivity was assessed using a toluene scintillator (toluene–PPO–POPOP). Three experimental filters containing D3112 DNA and two filters without DNA serving as a control for nonspecific binding were used for each sample. In none of the experiments deviation from the average radioactivity shown in the graph exceeded 5%. Hybridization was carried out under the conditions of D3112 DNA excess relative to phage-specific RNA on the filters. We demonstrated that an increase in the amount of [^{3}H]RNA in the hybridization mixture led to a proportional increase in the amount of filter-bound [^{3}H]RNA when 5–25 μg of DNA per filter was used. The amount of phage-specific RNA was estimated from the radioactivity of RNA–DNA hybrids, as the difference in radioactivity between the experimental and control samples.

RESULTS

Transcriptional Mapping of TP D3112 Early and Late Genes

[^{3}H]RNA hybridization with various restriction fragments of phage D3112 DNA allowed us to map early and late D3112 genes to different genomic regions and to estimate transcription efficiency in these regions during lytic development of the phage.

Figure 1 shows the map of TP D3112 and the set of DNA fragments obtained after treatment of the phage DNA with *Sac*II and *Eco*RI. These fragments (I–VII) were isolated from agarose gel and used for hybridization with RNA. There was a considerable excess of D3112 single-stranded DNA used for hybridization relative to the phage-specific RNA.

Figure 2 shows the results of hybridization of [^{3}H]RNA isolated at intervals after the thermal induction of prophage D3112*cts15* with D3112 DNA (Fig. 2a) or with fragments I (the *cI* gene) (Fig. 2b), II (the *cip1*, *A*, and *B* genes and genes from the nonessential region, hereinafter denoted by *X*) (Fig. 2c), or IV (nonessential genes, *C* genes, and the *ts47* locus) (Fig. 2c).

Figures 2d and 2e show the results of [^{3}H]RNA hybridization with DNA fragments V (genes controlling the morphogenesis of the D3112 phage head), VI, and VII (genes controlling the morphogenesis of the phage tail).

These data indicate that the early RNA synthesized during the initial 10–15 min of the D3112 phage lytic development was homologous predominantly to the D3112 DNA region with coordinates 1.1–5.8 kb, whereas late RNA synthesized afterwards was homologous to the region with coordinates 18–38 kb. Figure 2f summarizes the data on transcription of different D3112 genomic regions. As is seen from Fig. 2f, the

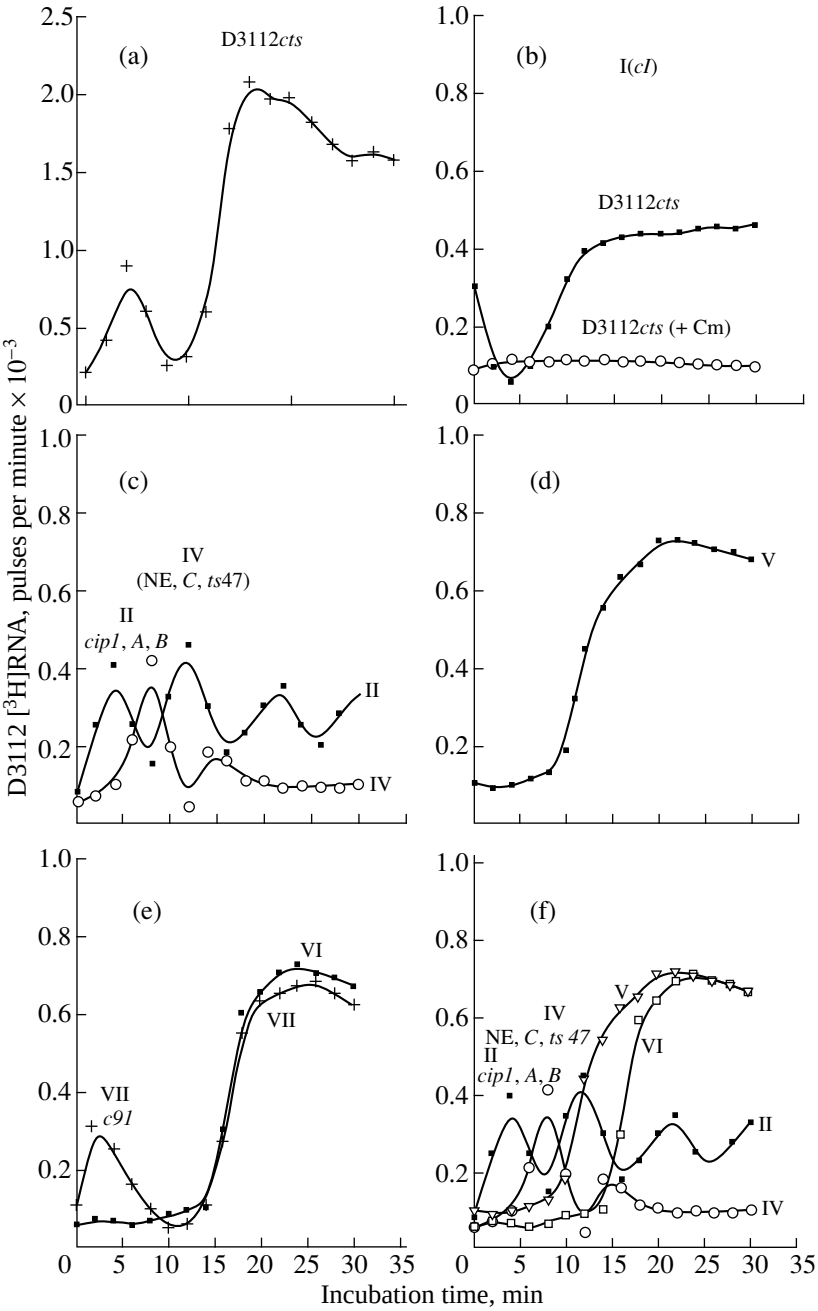

Fig. 2. Transcriptional mapping of phage D3112. Hybridization between [^{3}H]RNA and (a) denatured phage D3112 DNA immobilized on nitrocellulose filters or (b–f) DNA fragments was used to identify the transcripts. Transcription of different D3112 genomic regions was determined using hybridization with fragments (b) I (coordinates 0–1.1 kb; gene *cI* was mapped to this fragment), (c) II (1.1–5.8 kb; genes *cip1*, *A*, and *B*), IV (5.8–16 kb; genes *NE* and *C* and locus *ts47*), (d) V (22–29 kb; genes of the phage head morphogenesis), (e) VI (31–38 kb; genes of the tail morphogenesis), and VII (29–38 kb; early gene *c91* and genes of the tail morphogenesis). (f) The results from Figs. 2b–2e shown in the same graph *f* to compare the temporal patterns of transcription of different D3112 genomic regions. The fragments of D3112 DNA and (in parentheses) genes mapped to these regions are also indicated. Figure 2b shows the data on the transcription of the region with coordinates 0–1.1 kb when prophage D3112*cts* was induced in the presence of chloramphenicol (Cm). Figure 2e shows the presumed transcript of early gene *c91*.

phage genes were transcribed sequentially from the left to the right end of the phage genome.

The data shown in Fig. 2 also indicate that the region of early genes (*A* and *B*), which control DNA replication, is transcribed throughout the lytic development of the phage. The transcription of the *cI* repressor gene initially decreased and was completely suppressed within 4–6 min after induction. After this, it increased and remained about the same as before prophage induction. The efficiency of gene transcription decreased,

because the maximum transcription rate did not increase, whereas the number of phage DNA copies serving as templates for RNA synthesis increased constantly (see [9] and the present study). The genomic region with coordinates 29–32 kb was transcribed within the interval from 2 to 6 min after induction. Gene *c91*, which is involved in establishing the lysogenic state, has been mapped to this region [4]. An arrow in Fig. 2e indicates the *c91* gene transcript.

It was demonstrated earlier [9] that replication–transposition was entirely suppressed in TP D3112*cts15* carrying *ts* mutations in either *A* or *B* early genes (mutations *ts17* and *ts71*, respectively). The early RNA transcription in these phages did not differ from the early RNA transcription in phage D3112*cts15*, whereas the late RNA transcription was completely suppressed (see [15] and Figs. 3a, 3b). Results of hybridization with fragments of D3112 DNA confirmed that, between 0 and 10 min after thermoinduction of prophages D3112*cts15 ts17* and D3112*cts15 ts71*, genes located in the region with coordinates 1.1–5.8 kb were transcribed (there was hybridization with fragment II), while late genes were not transcribed (there was no hybridization with fragments V and VI; Figs. 3a, 3b). Analysis of D3112*cts15 ts104* and D3112*cts15 ts47* transcript incubated at 42°C yielded similar results (Figs. 3c–3e).

If the incubation temperature was decreased to 30°C after 6 min of PAO1 (D3112*cts15 ts104*) cultivation at 42°C (the replication–transposition was not blocked at 42°C in this mutant [9]), the transcription rate of late D3112 genes was considerably increased (Fig. 3d), and the titer of free phage increased by a factor of 100. A different behavior was observed for the phages mutant for genes *A* and *B*, in which replication was suppressed [9], and the phage with mutation *ts47*, in which replication was not suppressed [16]. Titers of these mutant phages did not increased when the incubation temperature was decreased to 30°C, and late genes were not transcribed.

Thus, the presence or absence of replication did not affect significantly the early gene transcription, whereas replication was necessary for late gene transcription.

The boundary between early and late genes is located in the region with coordinates 12–16 kb. Gene *C* and locus *ts47*, whose products are necessary for effective transcription of late genes, have been mapped to this region [15, 16].

Studying Regulation of Transcription of TP D3112 Carrying Polar Mutations in the Early Operon

To understand better the regulation of transcription of phage D3112 early genes, we studied transcription of D3112 polar mutants for early genes. We obtained the D3112 phages with the polar mutation that determined phenotype CIP^+ or CIP^-, depending on their location in the early operon. The absence or lack of the products of genes *A* and *B* was expressed as an absence of complementation of the corresponding *ts* mutants and a complete blockage of the phage DNA replication–transposition, which requires the *A* and *B* products [4, 9]. The CIP^+ and CIP^- phenotypes were detected by the effect of the heteroimmune TP B39 on the growth in bacteria PAO1, which are lysogenic for the phages studied [10]. Using the marker-saving method [21], we demonstrated that the genomes of the mutant phages retained intact *A* and *B* genes.

The general pattern of genome transcription in phage D3112*cts15* with phenotype $CIP^+A^-B^-X^-C^+$ considerably differed from the wild-type pattern (see Fig. 4a and Fig. 2, respectively). The former phage exhibited an intense transcription of the region between coordinates 1.1 kb and 5.8 kb of the physical map. According to the data on hybridization with fragment II (Fig. 4a), this region contains genes *cip1*, *A*, *B*, and *X*. In addition, in the mutant phage D3112*cts15* with phenotype $CIP^+A^-B^-X^-C^+$, normal expression of the *A* and *B* genes was blocked, which prevented DNA replication–transposition. A polar mutation in the early region is thought to block the transcription of the entire operon located to the right of the mutant site. Therefore, the observed transcription of the region between coordinates 1.1–5.8 kb (Fig. 4a) was probably related to the expression of gene *cip1*, which suppressed the growth of the heteroimmune TP B39.

In phage D3112*cts15* with phenotype $CIP^-A^-B^-X^-C^+$, the region between coordinates 1.1–5.8 kb was not transcribed, as can be seen from the data on hybridization with fragment II (Fig. 4b). The only difference between two phages was the presence or absence of the CIP phenotype; therefore, we may suggest that gene *cip1* is transcribed in the CIP^+ phage, but not in the CIP^- phage. The wave-shaped curve of the synthesis of the labeled RNA was an additional indication that gene *cip1* was transcribed in this genomic region between coordinates 1.1 and 5.8 kb in phage D3112*cts17* with phenotype $CIP^+A^-B^-X^-C^+$, because this curve resembled the pattern of the transcription of the *cip1* gene cloned on plasmid pBL3 [12].

The data on hybridization with fragment IV showed the transcription rate of the region with coordinates 6–12 kb increased; it reached the maximum on minute 6 after the D3112*cts15* phage with phenotype $CIP^+A^-B^-X^-C^+$ was induced (Fig. 4a). Apparently, this RNA corresponded to the transcripts of gene *C* and locus *ts47*. In the D3112*cts15* phage with phenotype $CIP^-A^-B^-X^-C^+$, this region was also transcribed (Fig. 4b). However, the transcription rate in the latter case was lower, with the maximum being reached only on minutes 10–12.

Hybridization with D3112 DNA fragments V and VI showed that late RNA synthesis was almost completely suppressed in both pages (Figs. 4a, 4b).

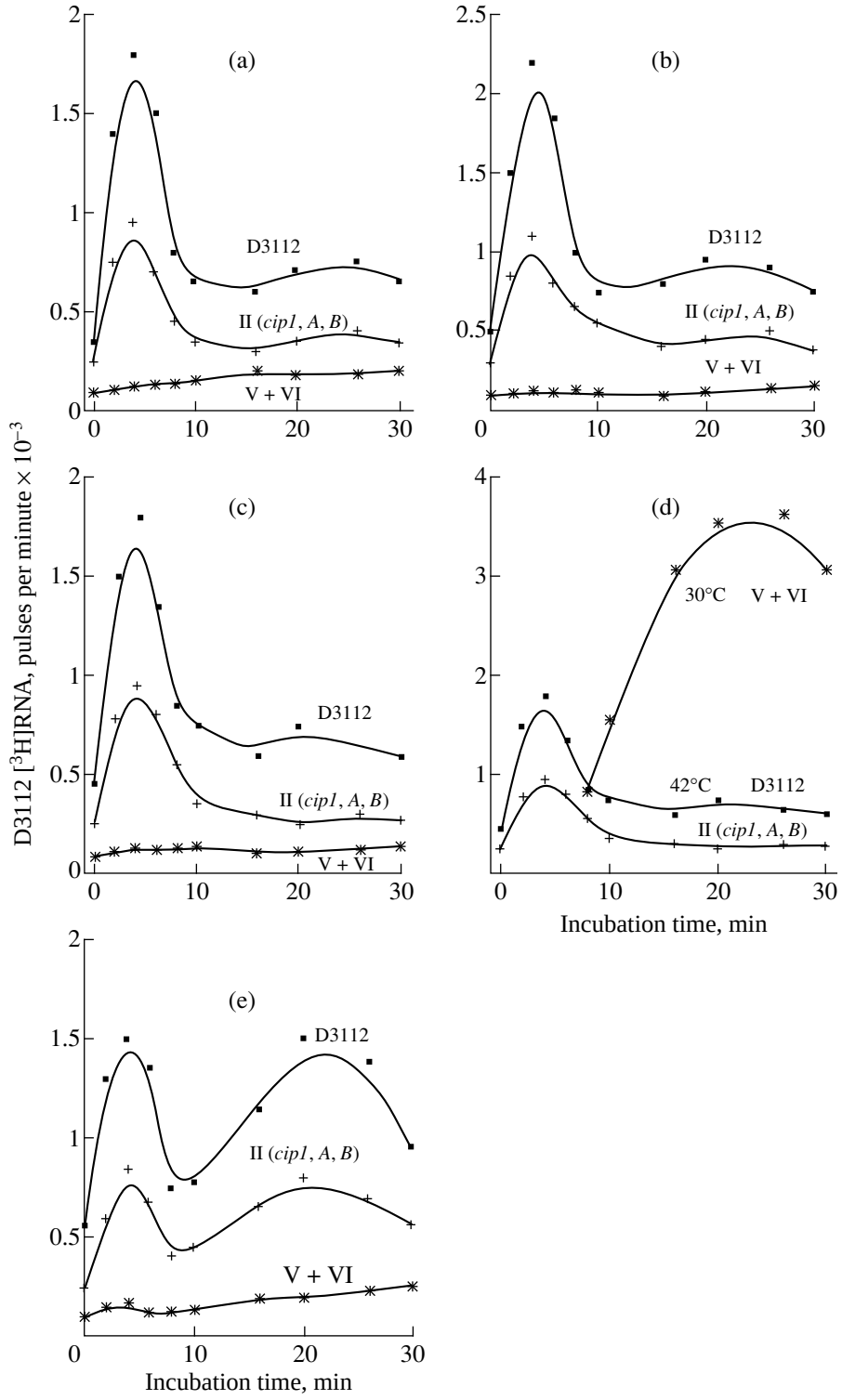

Fig. 3. Effects of *ts* mutations in early genes (a) *A* (*ts17*), (b) *B* (*ts71*) (both genes control replication–transposition), (c, d) gene *C* (*ts104*), and (*e*) locus *ts47* (controlling transcription of late genes) on the transcription of early and late genes of phage D3112. Transcription of early and late genes was determined using [^{3}H]RNA–DNA hybridization with denatured DNA of phage D3112 and DNA fragments containing early genes *cip1*, *A*, and *B* (fragment II) and the late genes controlling the head and tail morphogenesis (fragments V + VI). DNAs used for RNA–DNA hybridization are indicated. (d) After 8 min of incubation of the PAO1(D3112 *cts ts104*) cells at 42°C, the temperature was decreased to 30°C. The DNA species and the incubation temperature are indicated at each curve.

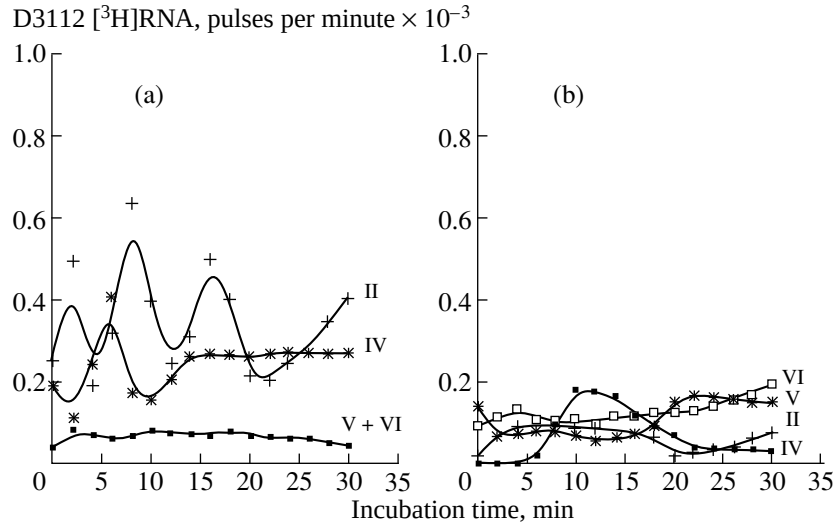

Fig. 4. Effects of polar mutations in the early genomic region of phages D3112*cts* with phenotypes (a) $CIP^+A^-B^-C^+$ and (b) $CIP^-A^-B^-C^+$ on the transcription of early (hybridization with fragments II and IV) and late (hybridization with fragments (V + VI) genes.

Regulation of the cI Gene Transcription

The TP D3112 gene *cI*, which controls the repressor (establishing and maintaining the lysogenic state), has been mapped between coordinates 0 and 1.8 kb of the TP D3112 physical map [4]. In the study [22], the nucleotide sequence of the left end (0–2.8 kb) of the D3112 genome was determined and an open reading frame was found between 1.0 and 0.3 kb. The position of this reading frame corresponds to nonhomologous region in heteroduplex molecules of genetically related heteroimmune D3112 TPs [23]. Therefore, we used D3112 DNA fragment I (0–1.1 kb).

As is seen from Fig. 5, inactivation of the thermolabile repressor in prophage D3112*cts15* considerably decreased the *cI* gene transcription, which was minimum on minute 4 after the phage induction. Afterwards, the amount of the *cI* transcript increased again; on minutes 6–8, it was equal or slightly higher than its amount before the phage induction. This amount of the *cI* transcript remained unchanged throughout the entire period of lytic development.

Little, if any, RNA homologous to the fragment I DNA was synthesized for 40 min in the presence of 25 μg/ml chloramphenicol. Therefore, the transcription of gene *cI* was regulated by a phage protein (or proteins) synthesized in the course of lytic development. We demonstrated that both negative and positive regulatory mechanisms existed.

The product of gene *cip1* is a negative regulator of gene *cI*. This is confirmed by the following data.

(1) Overexpression of gene *cip1* cloned on the multicopy plasmid pBL3 caused a complete suppression of the *cI* transcription (Fig. 5). Only gene *cip1* was cloned; hence, effects of other phage genes on the *cI* expression were excluded.

(2) Inactivation of the repressor immediately led to transcription of genes *cip1, A,* and *B,* with maximum synthesis of their RNAs coinciding with the minimum synthesis of the *cI* RNA.

(3) The first maximum of the *cip1* transcription in the D3112*cts15* prophage with phenotype $CIP^+A^-B^-X^-C^+$ (in which genes *A, B,* and *X* were not expressed) was observed on minutes 2–4 (Fig. 4a, hybridization with fragment II), i.e., at the same time as in the D3112*cts15* phage (Fig. 2c).

Regulation of the cip1 Gene Transcription

A complex mechanism regulated the transcription of early genes located in the region with coordinates from 1.1 to 5.8 kb in phage D3112*cts15* (Fig. 2c). We observed four minimums (on minutes 0, 8, 16, and 26) and four maximums (on minutes 4, 12, 22, and 30).

The D3112*cts15* mutant with phenotype $CIP^+A^-B^-X^-C^+$ exhibited transcription maximums for this genomic region on minutes 2, 10, and 18 after the prophage thermoinduction. In the CIP^- mutant phage, this region was not transcribed (Fig. 4b).

Transcription of gene *cip1* included in the *Hind*III–*Eco*RI D3112 fragment (coordinates 1.95–3.5 kb) [12] and cloned on a multicopy plasmid was similar to the transcription in the wild-type D3112*cts* phage and the D3112*cts15* phage with phenotype $CIP^+A^-B^-X^-C^+$. This unambiguously indicated that gene *cip1* was self-regulating and that its product negatively affected transcription of other early genes.

DISCUSSION

Thus, we studied the general pattern of the transcription of the *P. aeruginosa* D3112 TP genome in a homologous host. The transcription of this phage was similar to the transcription of Mu, a *E. coli* TP that is not genetically related to D3112 [15, 24, 25]. First, the lytic development of phage D3112 included two transcriptional waves differing in the rate of synthesis of the phage-specific RNA. The transcription was regulated by phage-specific proteins in both Mu and D3112 phages. The first transcriptional wave had a peak on minutes 3–5 of the D3112 lytic development. The decrease in DNA transcription rate on minutes 8–10 was determined by a phage-specific protein, because the transcription rate did not decrease if chloramphenicol was added. Gene *ner* serves as a negative regulator of early transcription in the *E. coli* Mu phage [7, 26]; in the D3112 phage, the homologically located gene *cip1* fulfils this function (see below). Transcription of early genes in D3112 is independent of replication–transposition of the phage DNA, which is also characteristic of phage Mu [20, 24]. Early transcription in D3112 is also independent on the expression of genes whose products were necessary for transcription of late genes. In phage Mu, gene *C* serves as a positive regulator of late gene transcription [27]. This gene has the coordinate 10 kb in the physical map. The homologically located D3112 gene *C* is also a regulatory gene, and its product is also necessary for late gene transcription [4, 15]. This confirms that genes *C* of the D3112 and Mu phages are functionally homologous to each other. To date, the mechanism of action of protein C in the D3112 phage is unknown. In the Mu phage, protein C serves as an antitermination factor for transcription initiated on late promoters [27].

Transcriptional mapping of the D3112 TP genome allowed us to determine the order and the rates of transcription of different genomic regions during lytic development. We found that early RNA of D3112 was mostly homologous to the phage genomic region with coordinates 1.1–18 kb (early phage genes, including *cip1*, *A*, *B*, *C*, and locus *ts47*). The region of early genes regulating replication–transposition exhibits the highest transcription rate between minutes 1 and 6. These genes are also transcribed later, although at a lower rate: while the amount of RNA homologous to these genes remains about the same, the number of copies of phage DNA increases. Similar relationships have been reported for early transcription of phage Mu DNA [20, 24].

Transcription of the D3112 phage DNA has some characteristic features. One of its early RNAs (expressed between minutes 1 and 3–4) is homologous to the genomic region with coordinates 29–32 kb, where late (morphogenetic) genes are located. However, gene *c91* involved in establishing the lysogenic state is located in the same region [4].

Note that the RNA homologous to the region with coordinates 29–32 kb is not detected in the absence of phage DNA replication or in the presence of chloramphenicol. Possibly, this transcript corresponds to the *c91* locus whose product stimulates lysogenization. This characteristic transcriptional pattern has not been found in phage Mu.

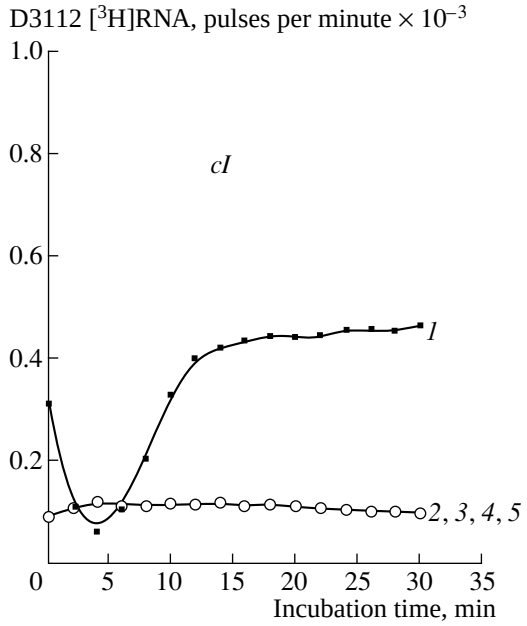

Fig. 5. The effect of the expression of gene *cip1* on the transcription of repressor gene *cI*. The transcription of *cI* was determined using [^{3}H]RNA–DNA hybridization with fragment I (coordinates 0–1.1 kb). [^{3}H]RNA was isolated from lysogenic bacteria collected at different times after thermoinduction (42°C). Transcription of gene *cI* during the development of phage D3112*cts* (*1*) in the absence of external effects, (*2*) in the presence of chloramphenicol, and (*3*) in the presence of plasmid pBL3 containing a cloned gene *cip1*, as well as phage D3112*cts* carrying polar mutations determining phenotypes (*4*) $CIP^+A^-B^-C^+$ and (*5*) $CIP^-A^-B^-C^+$.

Transcription of other structural genes of TP D3112 reached its peak 16–18 min after thermoinduction. Replication of phage DNA and expression of gene *C* and locus *ts47*, positive regulators of transcription of late D3112 genes, were necessary for transcription of this region. Late genes were not transcribed in phages Mu mutant for genes *A* and *B* [24].

The results of our study indicate that late genes of phage D3112 form two groups (genes of the phage head and genes of the tail), with an independent transcriptional unit corresponding to each group. RNAs homologous to these modules are synthesized independently of each other. Synthesis of the RNA homologous to the tail module begins on minutes 8–12 after thermoinduction, i.e., 4–6 min later than synthesis of the RNA homologous to the genes of the phage head. The same independent transcriptional units have been found in phage Mu [20, 28]. Apparently, this subdivision of the structural-gene region reflects the mutual independence of the head and tail morphogeneses [29].

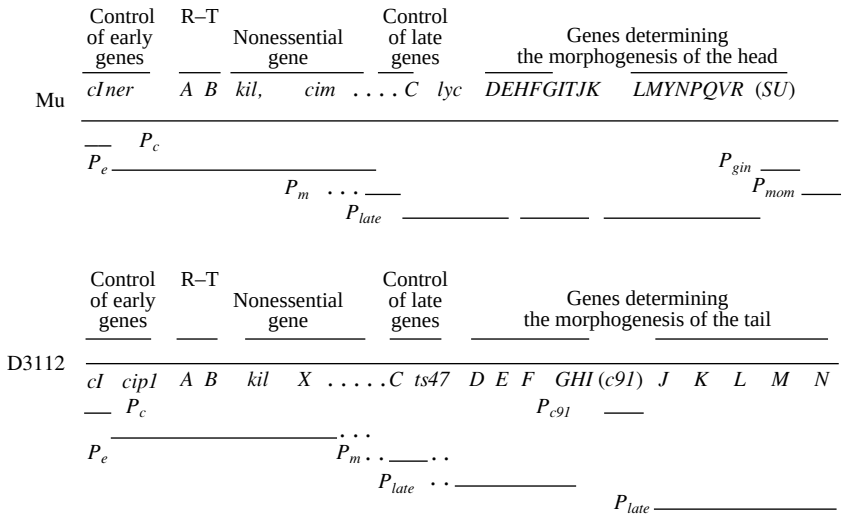

Fig. 6. Comparison of the transcriptional maps of the *E. coli* TP Mu [28] and *P. aeruginosa* TP D3112. Six independent transcriptional units were found in phage D3112. The transcripts are designated as P_c (the transcript of gene *cI*), P_e (early genes *cip1*, *A*, and *B*), P_{c91} (early gene *c91*), P_m (middle gene *C* and locus *ts47*), and P_{late} (independent transcripts of the late genes of the phage head and tail).

The transcriptional mapping of the TP D3112 genome carrying polar mutations in the early operon (whose locations determine which phenotype, CIP^+ or CIP^-, will the phage have) allowed us to determine more accurately the organization of the early genomic region. The data obtained indicate that genes *cip1*, *A*, and *B* are comprised in the same operon under a common promoter. By analogy with functionally similar promoters in other phages, we designated it as P_e. If this operon contains a polar mutation, the entire region to the right of the mutant site is not transcribed. For example, in the $CIP^+A^-B^-X^-C^+$ mutant phage D3112, the region of the *cip1* gene is transcribed, whereas little, if any, transcription is almost absent in the region located to the right of the coordinate 5.8 kb of the physical map. One exception is an insignificant increase in the synthesis of phage-specific RNA homologous to the middle genomic region (containing gene *C* and locus *ts47*) on minutes 4–6 after thermoinduction. The genes controlling the morphogenesis of the phage head (they are located to the left of the coordinate 29 kb), as well as the genes controlling the morphogenesis of the tail, are not transcribed in the $CIP^+A^-B^-X^-C^+$ mutant.

The polar mutant D3112 phage, which has the phenotype $CIP^-A^-B^-X^-C^+$, differs from the $CIP^+A^-B^-X^-C^+$ phage in an almost complete absence of transcription. Only an insignificant increase in the amount of phage-specific RNA corresponding to the middle genomic region is observed on minutes 4–6 of the phage development.

Gene *cI* is not transcribed in TP Mu either in the lysogenic state or during the lytic development [26]. In contrast, this gene in phage D3112 is transcribed. We analyzed its transcription using RNA–DNA hybridization. Thermoinduction of prophage D3112*cts* leads to inactivation of the thermolabile repressor. This is accompanied by a decrease in the synthesis of *cI*-specific RNA, which is minimum on minute 4 after the start of inactivation. After this, the amount of *cI*-specific RNA increases to approximate or exceed, on minutes 6–8, its amount before the prophage induction. We think that this characteristic feature of the regulation of the *cI* transcription is determined by the effect of a phage-specific protein (or proteins), *cI* RNA is synthesized at a constant, significantly decreased rate in the presence of chloramphenicol. Gene *cip1* possibly serves as a negative regulator of the transcription of gene *cI*. When studying the transcription of gene *cI* in $CIP^+A^-B^-X^-C^+$ and $CIP^-A^-B^-X^-C^+$ D3112 mutants, we found that, in the absence of protein Cip, the *cI* transcription rate increased by about two times compared to the initial rate, although it remained lower than in the wild-type phage. Experiments with plasmid pBL3 containing a cloned DNA fragment with no D3112 genes other than *cip1* [12] provided direct evidence that the product of this gene affected the transcription of the *cI* repressor gene.

Thus, gene *cip1* is involved in regulation of the *cI* gene transcription. Neither CIP^+ nor CIP^- polar D3112 mutant phage exhibited the decrease in the *cI* transcription rate between minutes 0 and 4 that was observed in the wild-type phage. Apparently, this was related to the lack of the products of certain early genes whose expression was blocked because of the polar mutation in the early operon.

The *cip1* gene product was shown [11, 12] to suppress both lytic development and lysogenization of bacteria by closely related heteroimmune TPs of *P. aeruginosa*. The degree of the suppression increased with an increase in the gene dose (number of copies of the D3112 genome) and was completely blocked in the presence of plasmid pBL3 containing a cloned *cip1*

gene [11, 12]. However, the lytic development of the D3112 phage itself was not affected by gene *cip1* (except when the gene was overexpressed in plasmid pBL3). This suggested that another gene negatively regulated the *cip1* gene expression.

Thus, the data obtained allowed us to construct a hypothetical transcriptional map of TP D3112 of *P. aeruginosa* (Fig. 6). According to this map, the genome of TP D3112 comprises six independent transcriptional units approximately corresponding to the module organization of the D3112 genome. The first of these units contains the gene encoding the phage repressor; the second unit, the early operon containing the *cip1*, *A*, and *B* genes; the third, the *C* gene and, possibly, the *ts47* locus; the fourth, the structural genes of the phage head; the fifth, the gene in which mutation *c91* is located; and the sixth, the structural genes of the tail.

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