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Regulation of Transcription of the Xp10 Genome in Bacteriophage-Infected *Xanthomonas campestris* pv. *oryzae*

YOU-DE LIAO,[1] JENN TU,[1] AND TSONG-TEH KUO[1,2]*

Institute of Botany[1] and Institute of Molecular Biology,[2] Academia Sinica, Taipei, Taiwan, Republic of China

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Results of in vivo studies showed that the transcription of the Xp10 genome in Xp10-infected cells shifted from rifampin sensitivity to rifampin resistance. Results of in vitro studies showed that a rapid reduction of rifampin-sensitive RNA polymerase activity coincided with a rapid increase of rifampin-resistant RNA polymerase activity in cell extracts with time after infection. Host and Xp10-encoded RNA polymerases were purified, and the transcripts from these two enzymes were hybridized to the restriction fragments of Xp10 DNA. The RNA probe generated by host RNA polymerase hybridized strongly to the leftmost 25% of Xp10 DNA and weakly to the rightmost 75% of Xp10 DNA. The RNA probe generated by Xp10 RNA polymerase hybridized strongly to the rightmost 75% of Xp10 DNA and weakly to the leftmost 25% of Xp10 DNA. Studies with ^{32}P-labeled RNA isolated at various intervals after infection did not reveal any evidence for early versus late differences in transcription.

During the development of Xp10, a virulent bacteriophage of *Xanthomonas campestris* pv. *oryzae* that is a phytopathogenic bacterium of rice, the regulation of the transcription of the Xp10 genome was observed. To study transcription regulation in this system, the host RNA polymerase from phage-infected and uninfected cells were purified and their properties were compared. It was found that the host RNA polymerase is modified by loss of the σ factor. After modification the ability to transcribe the Xp10 genome is completely lost (16). To clarify the mechanism involved in the increase of the rifampin-resistant RNA polymerase activity, an Xp10-encoded RNA polymerase was purified. The enzyme is composed of a single polypeptide of M_r = 96,000 (17). Based on the molecular weight, this enzyme is very similar to the phage-encoded enzyme isolated from coliphage T7 (5, 6) and T3 (4), *Pseudomonas* phage gh-1 (21), and *Salmonella* phage SP-6 (3). However, Xp10 RNA polymerase prefers denatured DNAs from Xp10, calf thymus, host bacterium, and poly(dA-dT) as templates (17), whereas the phage-encoded RNA polymerases isolated from T7, T3, gh-1, and SP-6 utilize only their own native phage DNAs as templates. The preference for denatured DNA and poly(dA-dT) is similar to that of coliphage N4-encoded RNA polymerase I and II. However, N4 RNA polymerase I is composed of a single polypeptide of M_r = 320,000 (12), and N4 RNA polymerase II is composed of two polypeptides with M_r = 40,000 and M_r = 30,000 (13, 22).

Because the mechanism involved in the turnoff of the transcription of Xp10 early genes and the properties of Xp10-encoded RNA polymerase are different from those of other phage systems (16) it is expected that the system involved in the regulation of the Xp10 genome should be different. We wanted to understand the role of host and Xp10-encoded RNA polymerase, which are involved in this system. We report here the time course for the development of rifampin-resistant transcription of the Xp10 genome in vivo and the altered pattern of Xp10 gene transcription in vitro with the two polymerases.

The host bacteria and phage used in this experiment were *X. campestris* pv. *oryzae* 604 and its phage Xp10, which were isolated in our laboratory. Host bacteria and phage Xp10 were grown in PS medium, as described previously (17). Preparation of host cells and Xp10-infected cells after infection have also been described (16, 17).

To study the change of transcription of the Xp10 genome during phage development, *X. campestris* pv. *oryzae* was grown in PS medium to a cell density of 5×10^8 cells per ml and then infected with phage Xp10 at a multiplicity of 10 phage particles per cell. Equal aliquots were removed at various intervals after infection. Rifampin (10 μg/ml) was added and incubated at 28°C until the culture without rifampin had lysed. Chloroform was added to the culture, and titers of phage particles were determined on PS soft agar overlays (7). Under these conditions the time required for lysis was 65 min. When rifampin was applied within 10 min after phage infection, phage formation was completely inhibited. After this initial period of infection, there was a gradual increase in the production of phage until the period between 60 and 90 min, when the culture began to lyse and the addition of rifampin had no effect on the yield of phage (Fig. 1A). Because the transcription system of the host bacterium is rifampin sensitive (16), the phage cannot grow in these cells in the presence of rifampin and the early stage of Xp10 development is inhibited. It appears that the host RNA polymerase is required for the transcription of early phage genes. However, the late stage of Xp10 development was not inhibited; therefore, the induction of rifampin-resistant transcription by Xp10 was expected.

To prove the expectation described above, cell extracts were prepared from cells at various time intervals after phage infection. The RNA polymerase activities for resistance and sensitivity to rifampin were measured (Fig. 1B). A rapid reduction of host RNA polymerase activity coincided with a rapid increase of Xp10-specific RNA polymerase activity. It shows the shift from the host RNA polymerase to the Xp10-specific RNA polymerase in normal Xp10 infections. When the cells were infected with Xp10 in the presence of chloramphenicol, no reduction in the activity of the host RNA polymerase and no increase in the activity of the Xp10-specific RNA polymerase were observed, indicating that protein synthesis after Xp10 infection is required to

* Corresponding author.

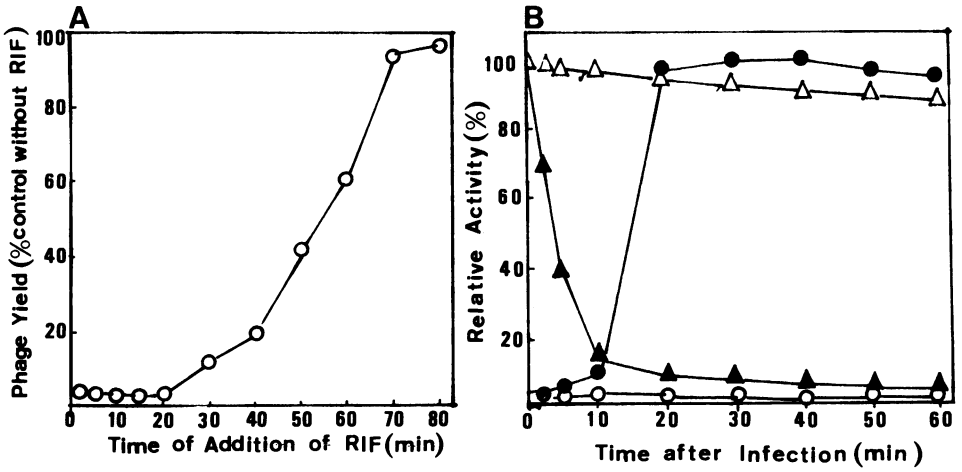

FIG. 1. (A) Effect of rifampin (RIF) on Xp10 production. Rifampin was added to a culture of *X. campestris* pv. *oryzae* at various periods after the addition of phage Xp10. A parallel culture was infected in the absence of rifampin. Incubation was continued for 65 min after the culture without rifampin had lysed. Chloroform was added to the culture, and the phage titer was assayed. The phage yield was determined by comparing the yield of phage from the cultures with rifampin with the yield of phage without rifampin. A rifampin-sensitive host cell was used. (B) Reduction of rifampin-sensitive RNA polymerase activity and appearance of rifampin-resistant RNA polymerase activity after infection of *X. campestris* pv. *oryzae* by Xp10. RNA synthesis was measured both in the presence and in the absence of rifampin in extracts prepared from Xp10-infected cells at various intervals after infection. Total enzyme activity minus rifampin-resistant activity was considered as rifampin-sensitive activity. Extracts were prepared from infected cultures grown either in the presence or absence of chloramphenicol (50 μg/ml). The rifampin-sensitive activity in the presence (△) or absence (▲) of chloramphenicol. The rifampin-resistant activity in the presence (○) or absence (●) of chloramphenicol.

accomplish the shutoff function and the induction of new Xp10-specific enzyme.

To study the transcription of the Xp10 genome by purified host RNA polymerase in vitro, the host RNA polymerase was purified to homogeneity as described previously (16) and was used to transcribe native Xp10 DNA. Labeling and isolation of RNA and the procedure for the hybridization of DNA and RNA have been described previously (16, 17). The ^{32}P-labeled RNA that was synthesized by host RNA polymerase was hybridized to denatured restriction fragments of Xp10 DNA that were blotted onto a membrane (Gene Screen; New England Nuclear Corp., Boston, Mass.), according to the instructions of the manufacturer (19). A physical map of *Xho*I, *Sst*II, *Bam*HI, and *Xba*I cleavage sites has been constructed previously (7). Xp10 DNA is cleaved by *Xho*I into 6 fragments, by *Sst*II into 10 fragments, by *Bam*HI into 8 fragments, and by *Xba*I into 7 fragments. The fragment orders from left to right for *Xho*I, *Sst*II, and *Bam*HI are shown in Fig. 2, the fragment order and for *Xba*I is shown in Fig. 3. The fragments generated by these restriction enzymes were used to determine the regions transcribed by RNA polymerase on the Xp10 genome. ^{32}P-labeled RNA synthesized by host RNA polymerase could hybridize to all *Xho*I fragments. However, the hybridization intensity of the *Xho*I B fragment, which comprises 25% of the left early region of Xp10 DNA, was much greater than those of the *Xho*I A, C, D, E, and F fragments, which comprise 75% of the right-hand late region of Xp10 DNA. When the RNA probe was hybridized to *Sst*II restriction fragments, the *Sst*II B and C fragments, which also comprise the left beginning of the Xp10 genome, were strongly hybridized. Hybridization to the *Sst*II G, A, F, E, and D fragments, which are located on the right side of the Xp10 genome, were weakly detected. A strong band *Sst*II-X, located above the *Sst*II B and C fragments, was undigested Xp10 DNA. Other *Sst*II fragments such as *Sst*II-H and -J were too small to be detected. For further confirmation of the results described above, the RNA probe was also hybridized to *Bam*HI restriction fragments. Because the fragment sizes of *Bam*HI-A and -A′ were almost identical, we could not distinguish them. When we referred to the results described above, we found that *Bam*HI-A and -E, at the left beginning of Xp10 genome, were strongly hybridized, and that *Bam*HI-A′, -F, -B, -C, and -D, at the right region of the Xp10 genome, were weakly hybridized. *Bam*HI-X was the undigested Xp10 DNA (Fig. 2).

Xp10-encoded RNA polymerase was purified to homogeneity as described previously (17) and was used to transcribe native Xp10 DNA. ^{32}P-labeled RNA that was synthesized by Xp10 RNA polymerase was hybridized with *Xho*I, *Sst*II, or *Bam*HI cleavage fragments of Xp10 DNA (Fig. 2). The RNA probe strongly hybridized with *Xho*I fragments A, E, C, D, and F; *Sst*II fragments A, F, E, and D; and *Bam*HI fragments A, F, B, C, and D. All of these fragments are located in the right-hand 75% of the Xp10 genome. Although Xp10 RNA polymerase was highly purified, hybridization to *Xho*I-B, *Sst*II-B and -C, and *Bam*HI-A′ and -E was also weakly detected.

Results of in vitro studies showed that the left-hand region of Xp10 genome is transcribed by host RNA polymerase and that the right-hand region of Xp10 genome is transcribed by Xp10 RNA polymerase. It is important to know how transcription occurs in vivo. For the isolation of RNA from phage-infected cells, *X. campestris* pv. *oryzae* was grown to 10^9 cells per ml in PS medium. To decrease the phosphate concentration, the culture was concentrated at one-half the cell density in a synthetic medium (15 g of sucrose, 1 mg of magnesium chloride, 10 mg of ferric chloride, 1 g of glutamate, 50 mg of cysteine, 21 g of Tris hydrochloride, 1 g of ammonium chloride, 1.0 g of potassium chloride, 0.22 g of calcium chloride, and 20 mg of potassium dihydrogen phosphate per liter). The phage grew normally under these conditions.

The culture was allowed to grow for 30 min, $^{32}PO_4$ (150

μCi/ml; New England Nuclear) was then added, and phage Xp10 was added at a multiplicity of 10 phage particles per cell. Incubation was continued at 28°C and 7.5-ml fractions were removed at intervals. The infection was stopped by the addition of an equal volume of cold buffer B (0.02 M Tris hydrochloride [pH 8.0]; 0.1 M $MgCl_2$, 0.1 mM disodium EDTA, 0.1 mM dithiothreitol) containing 0.1 M sodium azide, and the culture was kept on ice. When chloramphenicol (50 μg/ml) was used, it was added 5 min before Xp10 infection, and 7.5-ml fractions were removed for analysis. The RNA was extracted twice by the hot phenol method at 65°C, as described previously (1). The samples were then washed once with ethanol, dried in vacuo, and suspended with 0.2 to 0.5 ml of TE buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA) plus 0.1% sodium dodecyl sulfate. ^{32}P-labeled RNA isolated from Xp10-infected cells at various intervals after phage infection was hybridized to restriction fragments of Xp10 DNA. RNA isolated from cells 5 min after phage infection, which represents the early stage of phage infection, was hybridized to *Xho*I restriction fragments. The RNA probe strongly hybridized to *Xho*I-B and weakly with *Xho*I-A, -C, and -D. In the case of *Xba*I, the RNA probe was hybridized to all the *Xba*I fragments E, F, B, A, D, and C. However, the density of *Xho*I fragments E, F, and B was much stronger than those of *Xba*I fragments A, D, and C. *Xho*I-X was undigested Xp10 DNA. It seems that at an early stage of phage infection the left region of the Xp10 genome was strongly transcribed. The transcription of the right

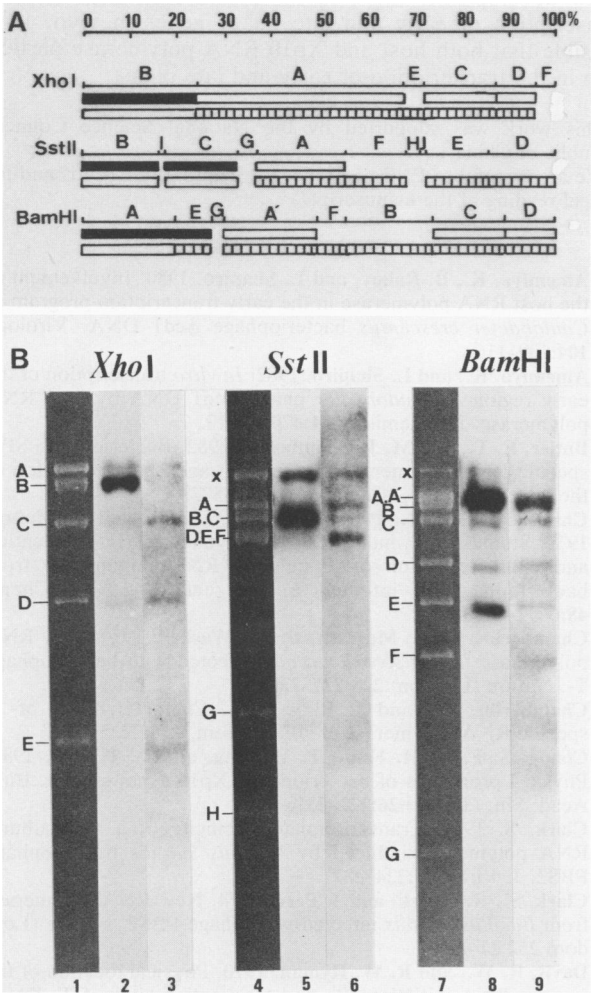

FIG. 2. Transcription map of host and Xp10 RNA polymerase on Xp10 DNA. Xp10 DNA was digested with *Xho*I, *Sst*II, and *Bam*HI. The digested Xp10 DNA fragments were fractionated in 0.8% agarose gel DNA fragments blotted on a membrane (Gene Screen; New England Nuclear) and were hybridized with a [^{32}P]RNA probe made with host and Xp10 RNA polymerases. (A) Restriction maps of *Xho*I, *Sst*II, and *Bam*HI. The closed boxes represent the region of Xp10 DNA transcribed by host RNA polymerase. The hatched boxes represent the region of Xp10 DNA transcribed by Xp10 RNA polymerase. The open boxes represent the region of Xp10 DNA transcribed weakly by host of Xp10 RNA polymerase. (B) Lanes 1, 2, and 3; lanes 4, 5, and 6; and lanes 7, 8, and 9 were the restriction fragments of Xp10 DNA that were produced by *Xho*I, *Sst*II, and *Bam*HI, respectively. The transcripts produced by host polymerase were used to probe lanes 2, 5, and 8; and transcripts produced by Xp10 polymerase were used to probe lanes 3, 6, and 9. Band X was undigested Xp10 DNA.

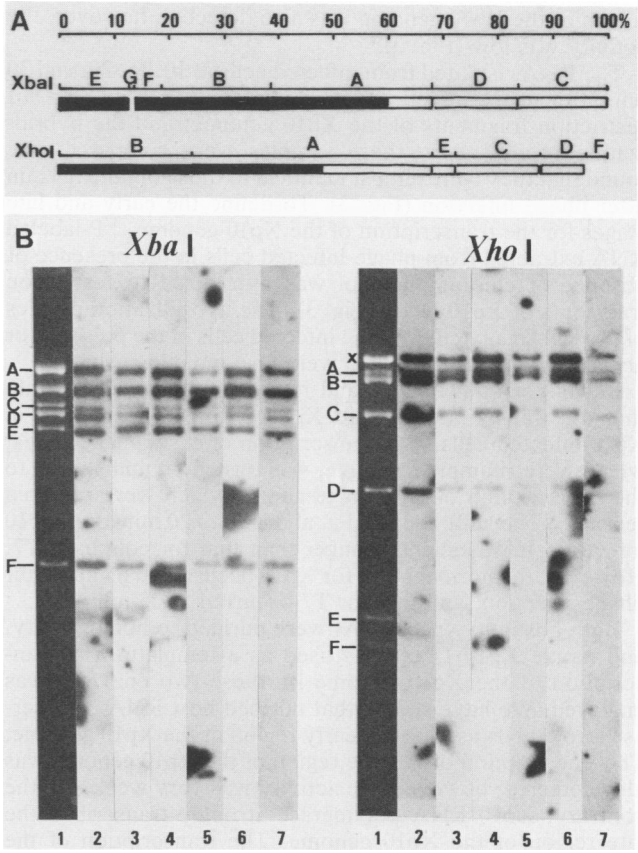

FIG. 3. Hybridization of in vivo ^{32}P-labeled RNA with Xp10 DNA restriction fragments blotted on membrane (Gene Screen; New England Nuclear) strips. (A) Restriction maps for *Xba*I and *Xho*I. The closed boxes represent the region of Xp10 DNA that was strongly hybridized by the RNA probes, which were isolated from Xp10-infected cells at various intervals after phage infection. The open boxes represent the region of Xp10 DNA weakly hybridized by RNA probe. (B) Lane 1, *Xba*I-digested Xp10 DNA fragments were fractionated on a 0.8% agarose gel; lane 2, transcription pattern of the RNA probe extracted from the cells 30 min after Xp10 infection in the presence of 50 μg of chloramphenicol per ml. The transcription pattern of the RNA probe extracted from the cells 5 min (lane 3), 10 min (lane 4), 15 min (lane 5), 20 min (lane 6), and 30 min (lane 7) after Xp10 infection in the absence of chloramphenicol is shown. Band X was undigested Xp10 DNA.

region of the Xp10 genome was also detected; however, the activity was low (Fig. 3).

The RNAs isolated from infected cells at 10, 15, 20, and 30 min after phage infection were hybridized to *Xba*I or *Xho*I restriction fragments of the Xp10 genome, and the hybridization profiles from these samples were compared. We found that they were almost identical to those obtained 5 min after phage infection (Fig. 3). To define the early and late stages for the transcription of the Xp10 genome, ^{32}P-labeled RNA extracted from phage-infected cells in the presence or absence of chloramphenicol was hybridized to restriction fragments of Xp10 DNA (Fig. 3). The hybridization profiles of the RNA isolated from the infected cells in the presence or absence of chloramphenicol were also indistinguishable.

Results of both in vivo and in vitro studies demonstrate that the transcription of the Xp10 genome is regulated in Xp10-infected cells. The transcription of early phage genes, which were rifampin sensitive, was turned off and shifted to the transcription of late phage genes, which were rifampin resistant. The shift occurred at about 10 to 20 min after Xp10 infection. It was slightly longer than that for coliphage T7. However, the normal burst for Xp10 occurred at 65 min after phage infection, and that for T7 occurred at 25 min (5).

Both host and Xp10 RNAs were purified to homogeneity, and native Xp10 DNA was used as a template. The transcription of the Xp10 genome by these two enzymes was analyzed. We have shown that purified host RNA polymerase strongly transcribe the early region of the Xp10 genome. The transcription of the late region of the Xp10 genome was also detected; however, the activity was very weak. On the contrary, Xp10 RNA polymerase strongly transcribed the late region of the Xp10 genome. The transcription of the early region of the Xp10 genome was also weakly detected. The transcription of phage early genes by host RNA polymerase has been reported for coliphage T7 and T3, *Pseudomonas* phage gh-1, *Caulobacter* phage φcd1, and *Salmonella* phage SP-6. Transcription by isolated host RNA polymerase has been shown to be highly specific (2, 10, 11, 14, 15). The bacteriophage-encoded RNA polymerases have been reported for coliphage T7 (5, 6) and T3 (4), *Pseudomonas* phage gh-1 (21), *Salmonella* phage SP-6 (3), *Bacillus subtilis* phage PBS2 (8, 9), and coliphage N4 (13, 22). The phage-encoded RNA polymerases from T7, SP-6, and PBS2 can specifically transcribe only the phage late genes (9, 15, 18). The phage-encoded enzymes from T3 and gh-1 can transcribe both early and late phage genes (11, 14). N4-encoded RNA polymerase transcribes the middle region of the N4 genome (22).

By using the DNA-RNA hybridization experiment, the stage of transcription of the Xp10 genome in vivo was studied. We found that the hybridization profiles from early and late stages were indistinguishable. Therefore, the stage of transcription of the Xp10 genome in vivo is not clear. In other bacteriophages such as T7, T3, gh-1, φcd1, and SP-6, there are stages for the transcription of early and late genes (1–3, 11, 14, 20).

Because transcription of the Xp10 genome in vivo was slightly different from that of other phage systems, the results from in vivo studies were further confirmed with those of other experiments. For the first experiment, the hybridization profile from cells grown in the presence or absence of chloramphenicol was compared; this was used to prove the existence of early and late stages of gene transcription in other phage systems. We found that the patterns from 5, 10, 20, 30, 40, and 50 min after infection were identical. For the second experiment, ^{32}P-labeled RNA from pulse-labeled cells harvested from early and late stages were also hybridized to restriction fragments of Xp10 DNA, and the hybridization profiles were compared (unpublished data). The hybridization was only detected at the samples from 5 and 10 min after infection; the samples from 20, 30, 40, and 50 min after infection were not detected. The transcription of the Xp10 genome became very low at late stages of phage infection. The hybridization profiles from 5 and 10 min after infection were also identical. In addition, host RNA polymerase transcribed the late region of the Xp10 genome, and Xp10 RNA polymerase transcribed the early region of the Xp10 genome. These data lead us to suggest that during Xp10 development there is no distinctive stage for the transcription of early and late phage genes in vivo. It is possible that both host and Xp10 RNA polymerase participate in the transcription of early and late phage.

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