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Expression of Phage Transcription in P2 Lysogens Infected with Helper-Dependent Coliphage P4

(satellite phage/sequence homology/chloramphenicol/ rifamycin/transactivation)

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ABSTRACT **The DNA of helper-dependent coliphage P4 and the DNA of its helper—P2—show no detectable sequence homology as measured by DNA·DNA hybridization. The lack of cross-hybridization permits direct analysis of P4 as well as of P2 transcription in P4-infected P2 lysogens by RNA·DNA hybridization. P4-transactivated P2 transcription can be detected around 20 min after P4 infection of the P2 lysogen and the rate (per infected cell) of that transcription becomes equal to that of the P4 transcription at the end of the latent period of P4. Furthermore, P4 transcription appears to be stimulated by the presence of the helper. Conceivably, P2 codes for a stimulator of P4 transcription. Rifamycin has been used to investigate the role of the host RNA polymerase during P4 transactivation of P2 transcription. The results exclude the participation of a P4-coded RNA polymerase and indicate that the original host RNA polymerase is responsible for the bulk of P4 and P2 transcription during transactivation.**

Satellite phage P4, originally isolated from an *Escherichia coli* strain K-235 (1), depends for its maturation on the head, tail, and lysis genes of a helper phage. P4 DNA replication and lysogenization, however, are independent of the helper (2). The help can be provided by the presence of a coinfecting phage such as P2 or by a repressed P2 prophage. In the latter case P2 immunity remains intact and the P2 prophage genome is not induced to replicate autonomously (3). Hence, P4 appears to activate the P2 prophage by a mechanism that bypasses P2 immunity (4). This event has been termed transactivation of the helper genome by P4 (5). The mechanism underlying this phenomenon remains unknown.

In this paper we use RNA·DNA hybridization techniques to study the expression of P2 and P4 transcription during transactivation. Separate analysis of P4 and transactivated P2 transcription in P4-infected P2 lysogens is made possible by the lack of sequence homology between P2 and P4 DNA.

MATERIALS AND METHODS

Bacterial and Phage Strains. E. coli HF4704 rif^s (C-1971) is a rifamycin-sensitive (permeable) derivative of HF4704 (nonsuppressor, host cell reactivation defective, thymine-requiring) (6) isolated and kindly provided by E. Ljungquist (7, 8). A rifamycin-resistant (RNA polymerase mutant) derivative of C-1971 was isolated by resuspending a 10-ml overnight culture in 0.1 ml of 0.1 M ethylenediaminetetraacetate followed by immediate plating of the cells on a LA plate (9) containing 100 μg of rifamycin per ml of agar. The cells were incubated for 2 days at 37° and a rifamycin-resistant colony picked and restreaked. This resistance must be due to a rifamycin-resistant RNA polymerase, since RNA synthesis in crude extracts of the C-1971 rif^r isolate is unaffected by the presence of rifamycin (unpublished results). A P2 lysogenic derivative of C-1971 or C-1971 rif^r was obtained by lysogenization with P2.

P4 *vir*$_1$ is a spontaneous mutant insensitive to P4 immunity (2) and P4 *vir*$_1$ *amA*$_1$ is a DNA-defective amber mutant isolated by Gibbs *et al.* (8).

Media and Chemicals. TPG-CAA, a Tris base minimal medium supplemented with casein amino acids has been previously described (10). Rifamycin SV was a generous gift from Dumex A/S and chloramphenicol was purchased from Sigma Chemical Co. [*Methyl*-^{3}H]thymine (specific activity 20 Ci/mmole) and [*6*-^{3}*H*]uridine (specific activity 15 Ci/mmole) were purchased from Amersham Radiochemical Centre, England. Nitrocellulose filters (Sm 11306) for RNA·DNA and DNA·DNA hybridization were obtained from Sartorius Membranefilters GmbH.

Infection Conditions and RNA Extraction. C-1971(P2), where (P2) indicates a strain lysogenic for that phage, was grown in TPG-CAA medium supplemented with 10 μg of thymine per ml at 37° with aeration to about 1×10^8 cells per ml and infected at the same temperature with P4 at a multiplicity of infection of about 10 (times, $t = 0$). Radioactive label was administered in pulses of 2 min at 10-min intervals by pipetting a 5-ml aliquot of the culture into a prewarmed growth tube (37°) containing 200 μCi [^{3}H]uridine. When pulses were performed in the presence of rifamycin, the drug was added 5 min prior to the addition of the label. Termination of the pulse, preparation of the RNA extracts, and RNA·DNA hybridization procedures were performed as described previously (11).

Strand Separation of P2 DNA was as described previously (11).

DNA·DNA Hybridization. The nitrocellulose membrane filter technique described by Denhardt (12) has been used. The filters contained 2 μg of P2, P4, or *E. coli* HF4704 DNA, respectively.

Abbreviations: PFU, plaque-forming unit; TPG-CAA, a Tris base minimal medium supplemented with casein amino acids.
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Preparation of Tritium-Labeled P2 and P4 DNA. E. coli HF4704 and HF4704(P2) were grown separately to a concentration of about 3 × 10^8 cells per ml at 37° in 25 ml of TPG-CAA supplemented with 10 μg of thymine per ml. The cells were collected by centrifugation and resuspended in 25 ml of fresh TPG-CAA supplemented with 2 μg of thymine per ml and 10 μCi of [^{3}H]thymine per ml. HF4704 was infected with P2 and HF4704(P2) with P4 (multiplicity of infection = 5). Two and a half milliliters of 0.1 M ethylenediaminetetraacetate were added to the cultures prior to lysis (at 25 min for P2 and 55 min for P4) to stop readsorption of phage to debris. The phages were collected (P2 at 60 min and P4 at 90 min after infection) and purified by two cycles of differential centrifugation at 25000 rpm for 2.5 hr in a Spinco 30 rotor. The phages were finally purified by CsCl equilibrium centrifugation. The specific activity of the purified phages was 1.2 × 10^{-5} cpm/plaque-forming unit (PFU) and 5.0 × 10^{-6} cpm/PFU for P2 and P4, respectively. Phenol extraction of phage DNA was performed as described previously (10).

RESULTS

The DNAs of P2 and P4 Do Not Cross-Hybridize. The result presented in Table 1 indicates that the genomes of P2 and P4 contain little, if any, sequence homology. This conclusion is also supported by a comparable lack of hybridization between P4 mRNA and P2 DNA or P2 mRNA and P4 DNA (11). This lack of homology permits separate analysis of P2 and P4 transcription in coinfections with P2 and P4 as well as in P4 infections of P2 lysogens by RNA·DNA hybridization techniques.

Expression of P4 Transcription in the Presence or Absence of the P2 Helper. We have previously shown that P4-transactivated P2 transcription can be detected between 10 and 20 min after P4 infection of a P2 lysogen (11). Under these conditions P2 transcription shows the same asymmetric strand distribution as that observed during lytic P2 infection, e.g., 95% of the P2 mRNA originates from the "heavy" and 5% from the "light" P2 DNA strand (11, 13).

TABLE 1. *P2-P4 DNA hybridization*

^{3}H-labeled phage DNA	cpm hybridized		
	to P2 DNA	to P4 DNA	to *E. coli* DNA
P2 DNA:			
Exp. 1	1076 (100%)	17 (1.7%)	28 (2.6%)
Exp. 2	923 (100%)	8 (0.9%)	13 (1.4%)
P4 DNA:			
Exp. 1	9 (0.4%)	2250 (100%)	0 (0%)
Exp. 2	26 (1.0%)	2701 (100%)	142 (5.3%)

Phenol-extracted tritium-labeled P2 or P4 DNA was sheared by forcing 0.3 ml of the DNA solution 20 times through a 1-ml tuberculin syringe (TOMAC, American Hospital Supply, Evanston, Ill.). This treatment yielded DNA fragments of less than 1 million daltons, as judged by sedimentation velocity analysis in sucrose gradients. The molecular weights of P2 and P4 DNA are 2.2 × 10^7 and 6.7 × 10^6, respectively (17). Aliquots of the sheared DNA preparations were subjected to DNA·DNA hybridization (12) with P2, P4, and *E. coli* DNA filters, respectively. Hybridization was performed at 65° for 15 hr. Under these conditions, the efficiency of hybridization was about 50% and the amount of hybridized label increased linearly with increasing inputs of labeled P2 as well as P4 DNA.

Here we include measurements of P4 transcription in a P4-infected P2 lysogen (presence of helper). As can be seen in Fig. 1*A*, the rate (per infected cell) of P4 transcription exceeds that of P2 transcription in the beginning of infection, but the rate of P4-transactivated P2 transcription increases rapidly and becomes equal to that of the P4 transcription at the end of the latent period (the latent period of P4 corresponds to about 65 min).

Cells infected with P4 in the absence of a helper survive the infection (4). Under such conditions the rate of P4 transcription does not increase but stays at its initial rate (Fig. 1*B*). This result suggest that the presence of the P2 helper has a stimulating effect on P4 transcription. A similar stimulation by P2 on the synthesis of P4-coded proteins has been observed by K. Barrett (personal communication).

Two classes of P4 mutants have been described, both of which are able to transactivate the P2 prophage under nonpermissive conditions (8). Mutants of one type (cistron *A* mutants) are blocked in DNA replication and also fail to make the poly(G)-synthesizing RNA polymerase (14). The result in Fig. 2 shows another aspect of the abnormal behavior of the cistron *A* mutants under nonpermissive conditions. As can be seen, the rate of transactivated P2 transcription increases normally, while the rate of P4 transcription, initially normal, decreases as the nonpermissive infection proceeds. See Fig 1*A* for comparison with P4*am*$^+$. This result could mean that part of the P4 genome is transcribed only in the presence of P4 DNA replication.

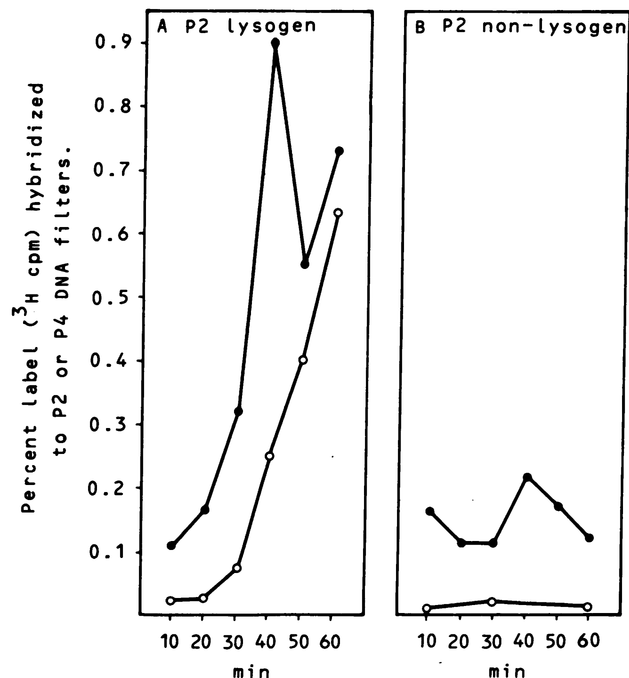

FIG. 1. P4 and P2 transcription. C-1971(P2) and C-1971 were infected with P4 *vir*$_1$. The infected cells were pulse labeled and RNA extracts were prepared as described in *Materials and Methods*. The [^{3}H]RNA in the extracts was subjected to hybridization with filters containing P2 and P4 DNA, respectively. The data represent the average result of two identical experiments. The percentages of hybridized label have been corrected for values obtained in the case of the extracts of uninfected bacteria (0.03% for P2 and 0.02% for P4). (*A*) C-1971 (P2) + P4; (*B*) C-1971 + P4. ● = P4 transcription; ○ = P2 transcription.

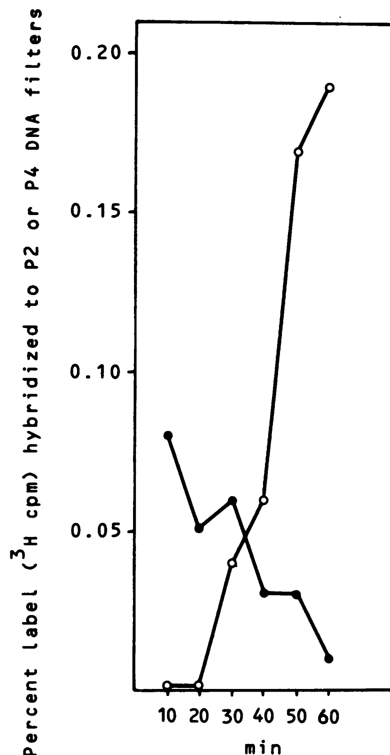

FIG. 2. P4 and P2 transcription in the absence of P4 DNA replication. C-1971(P2) was infected with P4 vir_1 amA_1 (nonpermissive infection) and pulse labeled, and RNA extracts were prepared as described in *Materials and Methods*. The [^{3}H]RNA in the extracts was subjected to hybridization with filters containing P2 and P4 DNA. The percentages of label hybridized to P2 DNA (0.03%) and P4 DNA (0.02%) from uninfected cell extracts have been subtracted. ● = P4 transcription; ○ = P2 transcription.

P4 Transactivation of P2 Transcription Depends on De Novo P4 Protein Synthesis. The search for conditional P4 mutants unable to transactivate P2 prophage has so far been negative in spite of the large number of mutants isolated (8). Conceivably transactivation could take place by some physical interaction between P4 DNA and P2 prophage, an event promoted solely by a host function(s). If that were the case, P4 transactivation of P2 transcription would be insensitive to chloramphenicol when added prior to P4 infection. Under such conditions P4 DNA replication is blocked (unpublished result). This block should not in itself interfere with transactivation, since it can take place in the absence of P4 DNA replication (Fig. 2 and ref. 8). The result presented in Table 2, however, shows that P4 transactivation of P2 transcription is blocked by 30 μg of chloramphenicol per ml when added to the cells 5 min before P4 infection. This result demonstrates that a P4-coded function(s) must participate in transactivation.

P4 and P4 Transactivated P2 Transcription Are Sensitive to Rifamycin. Rifamycin, a drug that is known to inhibit transcription in *E. coli* by affecting the *beta* subunit of the DNA-dependent RNA polymerase (15), has been used to investigate the role of the host RNA polymerase during transactivation. The yield of P4 particles is sensitive to rifamycin during the entire P4 latent period (unpublished results). This sensitivity cannot result from a block of P4 DNA replication, since addition of rifamycin 10 min after P4 infection does not arrest subsequent P4 DNA synthesis (14). The rifamycin sensitivity of P4 development must mean that at least one rifamycin-sensitive component, presumably the host RNA polymerase, is constantly involved in the transcriptional process leading to P4 maturation. Since P4 development requires P4 as well as P2 transcription, one or both of these processes may be sensitive to rifamycin. Consequently, it is possible that a rifamycin-resistant RNA polymerase may participate during transactivation, in addition to the host RNA polymerase (14). The experiment described in the *legend* to Fig. 3 was performed to investigate this possibility. The result shown in Fig. 3 demonstrates that both P4 and transactivated P2 transcription are sensitive to rifamycin. Hence, a P4-coded rifamycin-resistant RNA polymerase of the T7 type (16) can be involved neither in transactivated P2 transcription nor in P4 transcription—at least as a sole component. This finding is consistent with the notion that the rifamycin-resistant poly(G)-synthezising RNA polymerase described by Barrett *et al.* (14) from P4-infected cells participates in P4 DNA replication rather than in P4 or P2 transcription under *in vivo* conditions.

TABLE 2. *P4 transactivation of P2 transcription in the presence of chloramphenicol*

Sample	Percent label hybridized to "heavy" P2 DNA strands
+ P4, − Chloramphenicol	6.92
− P4, + Chloramphenicol	0.02
+ P4, + Chloramphenicol	0.04

C-1971 (P2) was grown and infected with P4 vir_1 as described in *Materials and Methods*. Chloramphenicol (30 μg/ml) was administered 5 min before infection. P4-transactivated P2 transcription was measured at 50 min after infection by addition of 50 μCi of [^{3}H]uridine during a 2-min pulse in a 5-ml sample. RNA extraction and RNA·DNA hybridization procedures were performed as previously described (11).

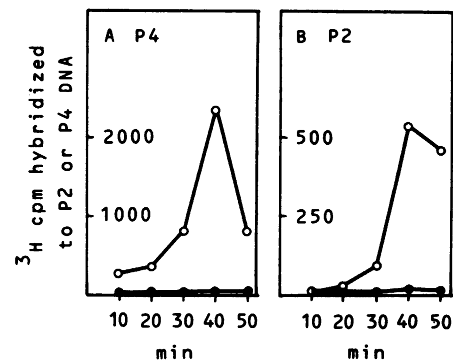

FIG. 3. P4 and P2 transcription in the presence of rifamycin. C-1971(P2) was infected with P4 vir_1 as described in *Materials and Methods*. Two 5-ml samples of the infected culture were removed at 10-min intervals and one of the samples was pretreated for 5 min with 100 μg of rifamycin per ml before both samples were pulse labeled for 2 min with 200 μCi of [^{3}H]uridine. Rifamycin (100 μg) was also present during the pulse labeling of the pretreated sample. RNA extraction and hybridization were performed as indicated in *Materials and Methods*. (*A*) P4 transcription: ○ = − rifamycin; ● = + rifamycin. (*B*) P2 transcription: ○ = − rifamycin; ● = + rifamycin.

TABLE 3. *P4 growth in a rifamycin-resistant strain*

Host	P4 yield (PFU/cell)	
	− rif	+ rif
C-1791(P2) rif^r	94	57
	36*	36*
C-1791(P2) rif^s	242	0.3

The cells were grown in LB broth (9) or TPG-CAA supplemented with 10 μg of thymine per ml to about 1 × 10^8 cells per ml at 37°. Two aliquots of each host culture were removed, one of which was pretreated with 100 μg of rifamycin (rif) per ml for 5 min before both samples were infected with P4 vir_1 at a multiplicity of about 5. After 20 min adsorption at 37° the infected cells were diluted 100 times into prewarmed medium (LB broth or TPG-CAA medium). The phage yield was measured at 135 min after addition of the phage.

* TPG-CAA medium.

In order to determine whether the rifamycin sensitivity is due to the *beta* subunit of the host RNA polymerase or a P4-coded rifamycin sensitive component, P4 growth was measured in a rifamycin-resistant strain with or without rifamycin present. As can be seen in Table 3, P4 growth is unaffected by rifamycin in the resistant strain. This result indicates that the original host RNA polymerase is responsible for the bulk of both P4 and P2 transcription during transactivation.

DISCUSSION

The lack of detectable sequence homology between P2 and P4 DNA suggests that the P4 genome may be little related to any of its potential helper genome. This notion is supported by the observation that P4 DNA replicates differently and independently of the helper genome (2). The cohesive ends of P2 and P4 DNA, however, are known to be identical (18). This identity most likely reflects a dependence of P4 DNA maturation on the maturation gene(s) of the helper.

The P4 transcription detected early in infection must include transcripts from genes responsible for P4 DNA replication (2), lysogenization (4), and transactivation. From the results presented here it is not clear whether the subsequent P4 transcription represents a new class of transcripts (late)— perhaps in addittion to the initial ones— or the early transcripts are transcribed at a higher rate late in infection. However, the first interpretation is likely to be correct, since the synthesis of P4-coded proteins can be divided into two classes, those synthesized early and those synthesized late in infection (K. Barrett, personal communication). The decreasing rate of P4 transcription during the nonpermissive infection with P4 vir_1amA_1 may reflect a shut off of the early class of transcripts, while the late transcripts fail to be made due to a possible requirement for P4 DNA replication. Furthermore, P4 late transcription appears to be stimulated by the presence of the helper (Fig. 1). Similarly, the presence of P2 increases the synthesis of P4 late proteins (K. Barrett, personal communication). Conceivably, P2 may direct the synthesis of a stimulator of P4 transcription.

The possibility that P4 codes for a special transactivating RNA polymerase (4)—as a sole transcribing component—is excluded by the present results. This finding is consistent with the *in vitro* properties of the P4-coded rifamycin-resistant RNA polymerase described by Barrett *et al.* (14). Hence, the original host RNA polymerase, modified or not, must be responsible for P4 as well as P2 transcription during P4 infection of a P2 lysogen.

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