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Bacteriophage φX174-Specific mRNA Synthesis in Cells Deficient in Termination Factor Rho Activity

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A previous report (Hayashi et al., Proc. Natl. Acad. Sci. U.S.A. **73**:3519-3523, 1976) indicated that in vivo bacteriophage φX174 mRNA's terminate after genes J, F, G, and H. However, termination at these sites is not stringent. To determine whether termination of φX174 transcription depends on rho factor activity, we introduced a temperature-sensitive rho mutation (*nitA*) into a φX174-sensitive host cell line and determined termination sites in wild-type and *nitA* cells. We found that (i) normal φX174 terminators were recognized in φX174-infected *nitA* cells, (ii) the rho mutation relieved polar effects caused by nonsense mutations in the phage genome or by chloramphenicol treatment of the host cells, and (iii) polarity was not caused by premature termination of transcription at the site of the polar mutation. RNA synthesis continued beyond the site to the first rho-sensitive site.

Bacteriophage φX174 in vivo mRNA can be fractionated into a number of discrete species by electrophoresis in polyacrylamide or agarose gels (4, 13). Hayashi et al. have mapped these RNA species by hybridization to restriction fragments of replicative form (RF) DNA (10). The resulting transcription map (Fig. 1) shows that the mRNA's starting before gene B terminate after gene J, F, G, or H. Another set of messages initiating before gene D terminate after gene J, F, or H. One species of message that starts before gene D transcribes the entire genome, reads through gene D, and terminates after gene J, resulting in a message larger than the entire phage genome. The in vivo transcription map indicates that there are termination sites after genes J, F, G, and H (T_J, T_F, T_G, and T_H, respectively). However, termination at these sites is not stringent. The efficiency of termination at sites T_J, T_F, and T_G is about 40 to 60%. The efficiency of termination is about 90% at the strongest termination site, T_H. Thus, the peculiar features of φX174 transcription, such as the presence of a gene transcript in more than one message and the synthesis of an mRNA larger than the entire genome, are consequences of inefficient termination events.

Two different transcription termination modes are known to exist. One mode is dependent on the rho termination factor (24), and the other mode is rho independent (for a review, see reference 1). The bacterial mutants designated *nit* (N-independent transcription) permit the transcription of lambda early genes beyond the rho-sensitive sites, even in the absence of the N gene product of lambda (16). One type of *nit* mutant, designated *nitA*, was shown to be a rho factor mutant by genetic (15, 16) and biochemical analyses (14, 28). We introduced a temperature-sensitive *nitA* mutation (*nitA702*[ts]) into a φX174-sensitive host cell line in order to determine whether termination of φX174 in vivo transcription depends on rho factor activity. We found that (i) normal termination sites of φX174 mRNA synthesis are recognized in *nitA* cells, (ii) the rho mutation relieves polar effects caused by nonsense mutations in the phage genome or by chloramphenicol treatment of the host cells, and (iii) polarity is not caused by premature termination of transcription at the site of the polar mutation. RNA synthesis continues to the first rho-sensitive site. Also, we observed pleiotropic effects of the *nitA* mutation on φX174 development.

MATERIALS AND METHODS

Construction of a φX174-sensitive *nitA* strain. The φX174-sensitive parent HF4704 (*uvrA thy*⁻) was converted to *ilv* by P1 transduction from KS666 (F⁻ *ilvC arg pro thi*) and subsequent negative selection by penicillin screening. The transductant HF4755 (HF4704 *ilvC*) was converted to HF4756 (HF4704 *ilvC argH*) by P1 phage (grown on KH1400 F⁻ *argH his ilv trpE9829*[am] *tyr*[am] *lacZ tonA tax bfe sup126*) and negative selection by penicillin screening (*argH* was added for other purposes and is not relevant to our findings). HF4756 was transduced with P1 (grown on HD163/F'16 F⁻ *nitA702*[ts] *trpE9829*[am] *tyr*[am] *thr recA*/F'16). HF4757 (HF4704 *ilv*⁺ *nitA702*[ts]

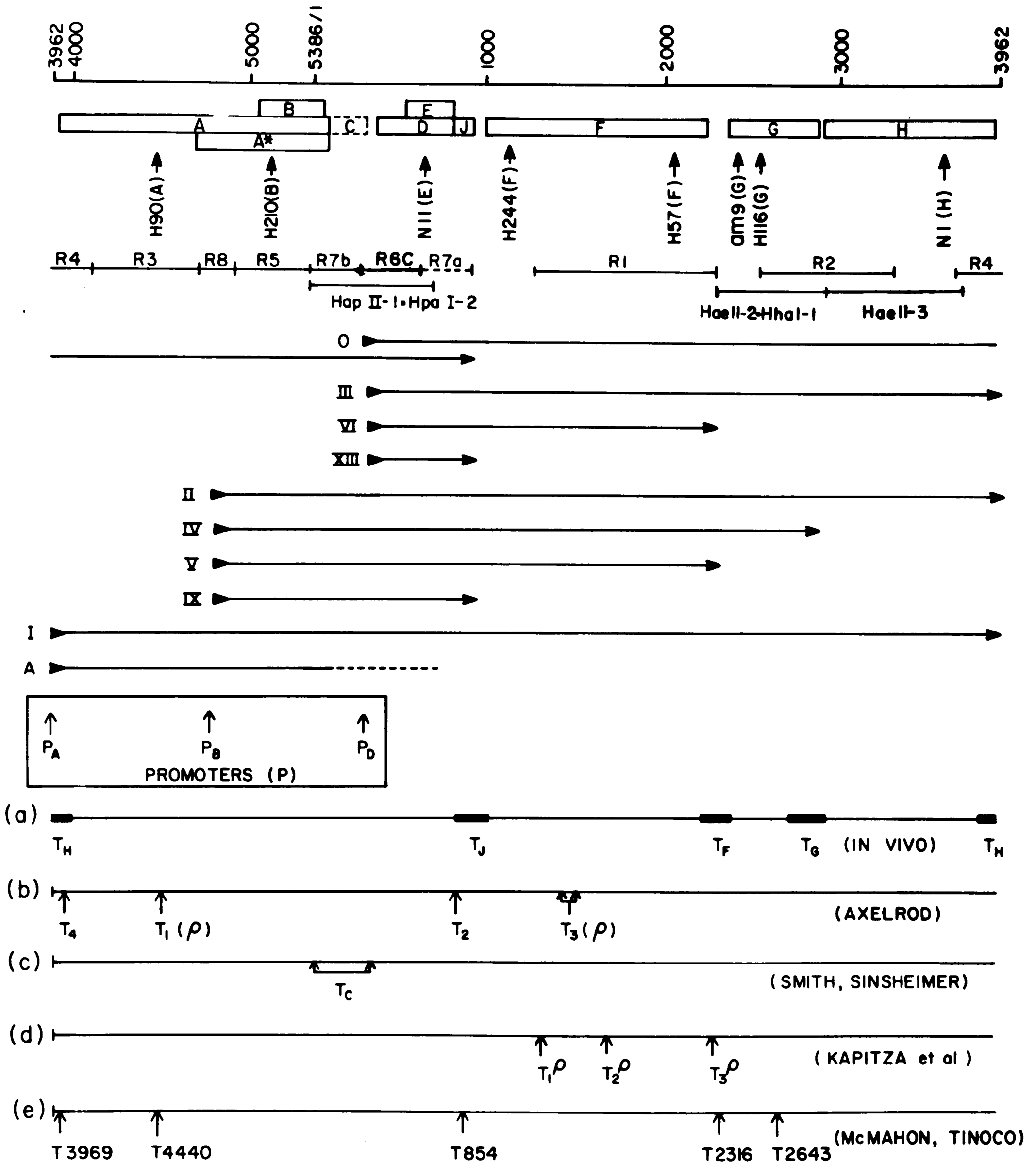

FIG. 1. *Map orientations of φX174 mRNA's (7). The top line shows nucleotide positions on the φX174 genome from the sequence of Sanger et al. (27). The second line (boxes) shows the gene arrangements. The map positions of the mutations of the φX174 mutants used in this paper are indicated under the boxes (12). R1 to R7b, HaeII-2-HhaI-1, and HaeII-3 represent the map positions of the restriction fragments used in this paper. The bars marked with Roman numerals I through VI, IX, and XIII and with the letters A and O represent the in vivo transcripts of φX174 mRNA's (10).* ▶— *and* —▶ *represent the initiation and termination sites, respectively.* P_A, P_B, *and* P_D *indicate the promoter sites of RNA polymerase (2, 10, 31). The initiation of peak I mRNA and mRNA A at* P_A *is tentative. mRNA A is functional unstable gene A mRNA. For details, see Hayashi et al. (10). Lines a through e indicate the deduced sites for transcription termination obtained from in vivo and in vitro experiments and from theoretical considerations. Line a, in vivo termination sites, from Hayashi et al. (10); line b, in vitro termination sites from Axelrod (2, 3); line c, termination sites from Smith and Sinsheimer (30–32); line d, termination sites from Kapitza et al. (18, 19) (ρ indicates rho-dependent termination); line e, termination sites from McMahon and Tinoco (21) (numbers represent the positions on the new map of Sanger et al. [27]). More detailed explanations of Fig. 1 are given in the text.*

argH) was isolated among the ilv^+ transductants. HF4757 was transduced with P1 (grown on KH6401 F^- *ilv argH trpE9829*[am] *tyr*[am] *thr thyA metE*). After negative selection by penicillin screening, HF4758 (HF4704 *ilv* $nitA^+$ *argH*) was obtained. The last step was needed to obtain $nitA^+$ wild type isogenic with the parental rho from which *nitA702* was derived (rho of K12 strain). We refer to HF4757 and HF4758 as *nitA* and wild type, respectively.

Media and phage mutants. The media and phage strains used in this paper have been described previously (8, 11). An amber mutant of φX174 in gene E (lysis) is referred to as wild-type phage.

Complementation. Complementation procedures have been described previously (17).

Heat treatment of host cells and infection procedure. An overnight culture of *nitA* cells was diluted with HFC medium. The diluted culture was grown at 30°C for about 2 h. When the exponentially growing culture reached an optical density at 660 nm of 0.3 to 0.35, it was transferred to 42°C and incubated for 2 h. The optical density of the culture stopped increasing after 90 min. An overnight culture of wild-type cells grown at 30°C was diluted with HFC medium and incubated at 42°C. These cells grew exponentially at 42°C. $CaCl_2$ (5 mM) and $MgCl_2$ (10 mM) were added to the heat-treated culture at the end of the heat treatment; 1 min after this addition, φX174 phage was added to the culture at a multiplicity of infection of 7 to 10 (zero time of infection). [^{3}H]uridine (20 to 50 μCi/ml; 0.2 to 0.4 μg/ml) or the four ^{14}C-labeled ribosides (0.5 μCi/ml; 0.3 μg of each per ml) were added to the culture between 15 and 20 min after infection. At the end of labeling, the culture was poured into a centrifuge tube containing frozen buffer (50 mM Tris-hydrochloride, pH 7.4, 5 mM EDTA, 200 μg of chloramphenicol per ml, 0.1 M NaN_3). The cells were collected by centrifugation for 2 min at 12,000 rpm in a Beckman JA-20 rotor.

Isolation of φX174 mRNA. The cell pellet derived from 20 ml of an infected culture was suspended in 4 ml of 2% sodium dodecyl sulfate containing 20 mM Tris-hydrochloride (pH 7.4) and 10 mM EDTA and blended with a Vortex mixer for 10 s. Water-saturated phenol was added to the lysate, and total nucleic acids were isolated by two successive phenol extractions and precipitation with ethanol in the presence of 0.3 M sodium acetate at −20°C. The isolation of φX174 mRNA from total nucleic acids was performed by DNA-RNA hybridization in formamide at 30°C as previously described (11), except that sodium dodecyl sulfate was omitted from the hybridization mixture. Isolated φX174 mRNA was kept in 70% formamide and heated at 60°C for 5 min before electrophoresis.

Gel electrophoresis of φX174 mRNA. A 1.75% agarose gel containing 6 M urea was prepared by the method of Rosen et al. (25). The gel was 6 mm in diameter and 15 cm long. RNA samples were electrophoresed for about 12 h at 2 mA/tube and 4°C. Gels were fractionated into 1-mm slices, which were soaked in 6 ml of toluene-based scintillation fluid containing 5% Protosol and 0.25% water. The direction of migration of the nucleic acids in the figures below was from left to right.

Hybridization of φX174 mRNA to restriction fragments. Total φX174 mRNA or specific fractions of φX174 mRNA eluted from gels after electrophoresis were hybridized to denatured restriction fragments as described previously (10). Isolation of some of the restriction fragments used in this paper has been described previously (10). pACYC 184 plasmid carrying the R1 fragment or the R6c fragment at the *Eco*RI site was a gift from K. Buckley in our laboratory. The fragments which covered genes G and H (designated *Hae*II-2-*Hha*I-1 and *Hae*II-3, respectively, in Fig. 1) were prepared in the following way. RF DNA was digested with *Hae*II, and *Hae*II-2 and *Hae*II-3 were isolated. *Hae*II-2 was digested with *Hha*I, and the largest fragment (*Hae*II-2-*Hha*I-1) was isolated.

RESULTS

Development of φX174 was inhibited in *nitA* cells. *nitA* cells and wild-type cells in tryptone broth were incubated at 42°C as described above. This procedure insured inactivation of rho factor (16). These cells were infected with wild-type phage, and burst sizes were measured. Wild-type cells produced about 30 phage per cell, whereas *nitA* cells had a lower burst size, about 5 phage per cell. We concluded that the *nitA* mutation affected φX174 development.

Normal termination sites of φX174 mRNA synthesis were recognized in *nitA* cells. Infected cells were labeled with [^{3}H]uridine as described above. The total incorporation of radioactivity in RNA (including host RNA and φX174 mRNA) in *nitA* cells was about 10% of the total incorporation in wild-type cells. When the labeled total RNA was hybridized to either *Escherichia coli* DNA or φX174 RF DNA, the ratios of *E. coli* RNA to φX174 mRNA in the two types of cells were almost identical. This indicated that syntheses of host and φX174 RNAs were inhibited to the same extent in *nitA* cells.

Labeled φX174 mRNA was isolated by hybridization and was analyzed in agarose-urea gels (Fig. 2). As reported previously (10, 13), wild-type cells synthesized several discrete sizes of φX174 mRNA's (Fig. 2b), and the overall pattern of these RNAs synthesized at 42°C was very similar to the pattern of the RNAs synthesized at 37°C. Peaks identified by Roman numerals refer to the RNA species in Fig. 1. φX174 mRNA's synthesized in *nitA* cells spanned size ranges similar to the size ranges of φX174 mRNA's synthesized in wild-type cells (Fig. 2a). Most of the RNA species found in wild-type cells existed in *nitA* cells. No preferential synthesis of larger RNA was found in *nitA* cells. Even shorter pulse-labeling of RNA did not change the RNA size distribution (data not shown). However, background counts across the gel profile were higher in RNA preparations from *nitA* cells. These background counts consisted of φX174

mRNA's not migrating as discrete peaks. RNAs from uninfected cells processed as RNAs from infected cells did not migrate in any region of the gel (data not shown). The slowly migrating peaks (labeled S·SDNA on the figures) consisted of single-stranded DNA derived from mature phage and replicative intermediates of DNA (data not shown). These peaks existed in wild-type cells infected with wild-type phage. Phage development in *nitA* cells was inhibited severely, and no significant amount of single-stranded DNA was recovered by hybridization and gel electrophoresis. The presence of background counts across the gel profile and the relatively small amounts of the larger RNA species (peaks I through IV) in *nitA* cells sometimes made it difficult to distinguish these larger species. To confirm that these peaks in *nitA* cells had cistron contents similar to those in wild-type cells, we designed an experiment to hybridize these RNAs to restriction fragments. However, it was impractical to collect sufficient radioactive material from each peak of RNA prepared from infected *nitA* cells because of poor incorporation of [^{3}H]uridine. Therefore, the [^{3}H]RNAs eluted from peaks I through IV were pooled, and the cistron contents of the pooled RNAs were determined (Table 1). It is evident that the higher-molecular-weight RNAs in *nitA* cells had cistron contents very similar to the cistron contents of the corresponding RNAs in wild-type cells (Table 1, samples 1 and 2).

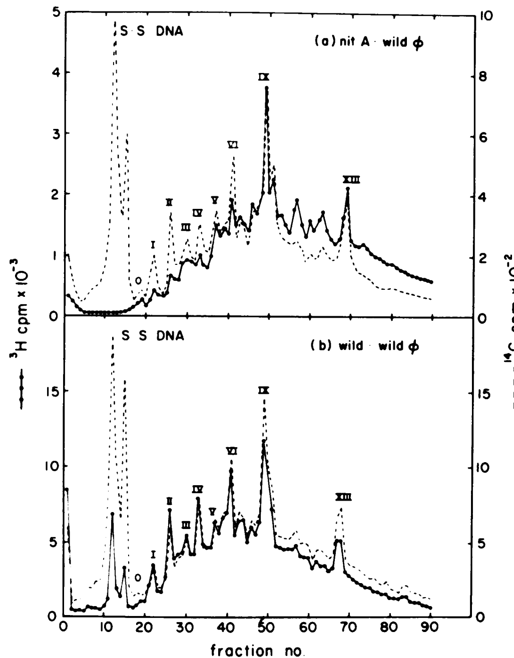

FIG. 2. *Size distribution of φX174 mRNA's in nitA cells and wild-type cells. φX174-specific mRNA's labeled with [^{3}H]uridine were isolated from nitA cells (a) and wild-type cells (b), as described in the text. These RNAs were mixed with φX174 mRNA labeled with ^{14}C-ribonucleosides isolated from HF4704 cells infected with wild-type phage (HF4704 is the parent strain of nitA$^-$ and nitA$^+$ cells) and grown at 37°C. RNA preparations were analyzed by agarose-urea gels as described in the text. S·S DNA, Single-stranded DNA derived from mature phage and replicative intermediates of DNA.*

nitA mutation released polarity induced by a nonsense mutation. Polar mutations are characterized by their capacity to inactivate both the cistron in which they are located and cistrons promoter distal to the site of the mutation. Nonsense mutations located near the amino-terminal portion of a cistron tend to be more polar than mutations near the carboxyl-terminal end. Imai has observed that *nitA* mutations relieve polarity caused by nonsense mutations in bacterial tryptophan and lactose operons (unpublished data). Polar mutants have been found among amber mutants of φX174 and the closely related phage S13 (13, 33, 34; Hayashi and Hayashi, unpublished data). Amber mutations mapping close to the N-terminal region in gene F cause reduced synthesis of gene G and H

TABLE 1. *Cistron contents of larger RNAs (region from peak I to peak IV)*[a]

Sample	Cell type	Infecting phage	% Hybridized to restriction fragments						
			R5 (B)[b]	R6c (D)	RI (F)	*Hae*II-2–*Hha*I-1 (G)	*Hae*II-3 (H)	RF	Background[c]
1	Wild type	Wild type	5.0	4.8	21.0	12.7	7.9	48.3	(0.21)
2	*nitA*	Wild type	4.9	5.2	22.1	11.9	6.8	46.6	(0.25)
3	*nitA*	H244	4.2	4.6	23.1	10.8	7.2	47.7	(0.26)

[a] φX174 total mRNAs were isolated from cells infected with phage at 42°C and fractionated in agarose gels. The RNAs migrating in the region from peak I to peak IV in Fig. 2a and b and Fig. 3a were eluted as described in a previous paper (10). RNA was precipitated with ethanol and hybridized to the fragments. The input radioactivity of the RNA used was about 3,000 cpm.

[b] Letters in parentheses indicate genes.

[c] Background value (no DNA in hybridization mixture) was subtracted.

proteins, whereas amber mutations mapping distal to the N terminus allow almost normal synthesis of gene G and H proteins. Similarly, amber mutations near the N-terminal region in gene G are polar to gene H.

We tested the effects of the *nitA* mutation on polarity in φX174 genes F, G, and H in the following three ways: (i) by complementation among mutants, (ii) by hybridization of total φX174 mRNA to the restriction fragments covemg the area downstream from the mutation sites, and (iii) by determining the size distribution of φX174 mRNA.

In the complementation tests, the effect of polarity was observed as a reduced burst size of phage, and recovery of the initial burst size indicated release from polarity. Table 2 shows the results of complementation tests among nonsense mutant phages in *nitA* and wild-type cells. When a polar mutant in gene F (H244) was complemented with gene G mutants (am9 and H116) or with a gene H mutant (N1) in wild-type cells at 42 or 30°C or in *nitA* cells at 30°C, the burst size was reduced compared with the burst size in a complementation test with a gene B mutant (H210) (gene B was not influenced by the polarity existing among genes F, G, and H; therefore, complementation with gene B mutants served as a control). The increased burst size was recovered when the identical complementation test was performed in *nitA* cells at 42°C. When a nonpolar gene F mutant (H57) was used for these complementation tests, we observed no significant reduction in burst size in the complementation tests with am9, H116, and N1. Comparisons of burst sizes in *nitA* and wild-type cells are shown in Table 2. The significant increase in burst size in complementation tests with the polar gene F mutant in *nitA* cells at 42°C indicates that the *nitA* mutation effectively rescued the polarity caused by the nonsense mutation.

A similar situation was observed when polar mutants in gene G (am9 and H116) were complemented with a gene H mutant (N1). Burst size reductions in the complementations in wild-type cells at 42°C were recovered effectively in *nitA* cells. However, complementation tests of these polar gene G mutants with the gene H mutant at 30°C produced higher burst sizes in *nitA* cells than in wild-type cells. The reason for this observation is not clear, but release of gene G polarity may be affected more strongly by the residual *nitA* phenotype, which may exist in *nitA* cells at 30°C. Finally, the wild-type phage in these complementation tests (assayed with the su⁻ indicator bacteria HF4704 and HF4757) was less than 0.2% of the total burst, and no

TABLE 2. *Complementation of polar and nonpolar mutants in nitA and wild-type cells (burst size)*[a]

Phage	Complementation at 42°C[b]			Complementation at 30°C		
	Burst size in:		Ratio of *nitA* value to wild-type value	Burst size in:		Ratio of *nitA* to wild-type value
	nitA cells	Wild-type cells		*nitA* cells	Wild-type cells	
H244 (F polar) × H210 (B)	3.9	4.7	0.8	41.4	40.4	1.0
H244 × am9 (G)	1.1	0.06	18.3	2.2	0.7	3.1
H244 × H116 (G)	6.7	0.09	14.4	4.3	2.8	1.5
H244 × N1 (H)	2.1	0.2	10.5	4.8	3.0	1.6
H57 (F nonpolar) × H210 (B)	0.9	1.9	0.5	21.4	27.0	0.8
H57 × am9 (G)	0.8	2.3	0.3	21.2	19.6	1.1
H57 × H116 (G)	3.2	7.1	0.5	20.0	22.6	0.9
H57 × N1 (H)	0.9	5.5	0.2	84.4	59.7	1.4
am9 (G polar) × H210 (B)	2.8	4.9	0.6	30.8	22.6	1.4
am9 × N1 (H)	1.2	0.4	3.0	7.8	1.0	7.8
H116 (G polar) × H210 (B)	2.4	4.3	0.6	25.1	20.9	1.2
H116 × N1 (H)	3.5	0.8	4.4	13.9	2.1	6.6
H57 (F) × H244 (F)	<0.05	<0.05		0.3	0.1	3.0
am9 (G) × H116 (G)	<0.05	<0.05		0.05	0.1	0.5
Wild type	5	30	0.2	173	200	0.9

[a] Complementation tests were performed in cells grown in tryptone medium. Cultures were grown at 30°C, and a portion of each culture was heat treated at 42°C as described in the text. The remaining portion was kept at 30°C. Burst sizes were measured with the indicator bacterium CR 63.1 (su⁺) (12).

[b] Complementation tests involving am9 were at 40.5°C.

enhancement of recombination in *nitA* cells was observed.

The amounts of the gene F, G, or H message in wild-type and *nitA* cells infected with polar mutants or with wild-type phage were determined by hybridizing total φX174 mRNA to appropriate DNA fragments (Table 3). Gene B DNA (R5) and RF DNA were used as controls. When wild-type cells were infected with a polar gene F mutant (H244), syntheses of gene G and H messages were reduced to 22 and 27%, respectively, of the syntheses in wild-type cells infected with wild-type phage. The am9 mutation caused a reduction in the gene H message to 18% of the level in cells infected with wild-type phage. However, when *nitA* cells were infected with H244 or am9 phage, we observed considerable recovery in the syntheses of gene G and H messages. *nitA* cells synthesized about 70% of the gene G and H messages found in wild-type cells infected with wild-type phage. These observations indicated that the *nitA* mutation was responsible for the release from polarity.

Figure 3 shows that the release from polarity was related to the species of mRNA synthesized in *nitA* cells. As shown previously (10), wild-type cells infected with a polar gene F mutant (H244) cannot synthesize larger RNAs, which contain messages for genes G and H (Fig. 3b, peaks I through IV). The synthesis of larger RNAs was stimulated in *nitA* cells (Fig. 3a). Table 1 shows that these RNAs had cistron contents similar to those in wild-type infected cells. We interpret this to mean that the larger RNAs were translated to relieve the polarity, although these RNAs were not as discrete in size as in the normal infection process. A gene G mutant (am9) which was polar to gene H (Table 2) could synthesize peak I, II, and III RNA (in which gene H RNAs were included) in *nitA* cells but could not make these RNA species in wild-type cells (Fig. 4a and b). Nonpolar mutants in gene F could synthesize all RNA species in both *nitA* and wild-type cells (data not shown).

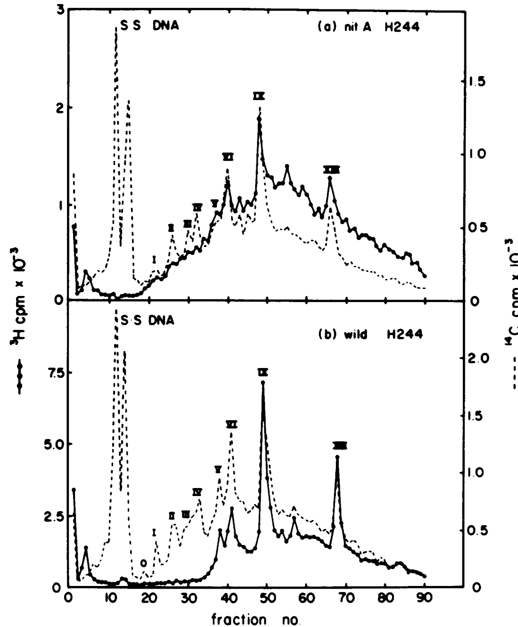

FIG. 3. *Size distribution of φX174 mRNA's in cells infected with a polar gene F mutant. [³H]uridine-labeled φX174 mRNA's from H244 (gene F polar mutant)-infected nitA cells (a) or wild-type cells (b) were mixed with φX174 mRNA labeled with ¹⁴C-ribonucleosides isolated from wild-type cells infected with wild-type phage at 42°C and were subjected to electrophoresis in agarose gels. S·S DNA, Single-stranded DNA derived from mature phage and replicative intermediates of DNA.*

TABLE 3. *Transcription of genes F, G, and H in wild-type and nitA cells infected with polar mutants*[a]

Host	Phage	% of RNA hybridized to restriction fragments					
		R5 (B)[b]	R1 (F)	*Hae*II-2-*Hha*I-1 (G)	*Hae*II-3(H)	RF	Background[c]
Wild type	Wild type	9.1 (100)[d]	6.6 (100)	2.7 (100)	1.7 (100)	50.0	(0.32)
	H244	10.8 (118)	5.2 (79)	0.62 (22)	0.46 (27)	51.4	(0.21)
	am9	9.9 (108)	5.3 (80)	2.2 (81)	0.32 (18)	47.5	(0.31)
nitA	Wild type	8.7 (100)	4.8 (100)	2.0 (100)	1.2 (100)	49.6	(0.35)
	H244	9.2 (105)	3.8 (79)	1.4 (70)	0.82 (68)	51.0	(0.31)
	am9	8.9 (102)	5.0 (104)	1.6 (80)	0.88 (73)	49.1	(0.36)

[a] φX174-specific total mRNA's labeled with [³H]uridine were isolated and hybridized to restriction fragments representing each gene as described in the text (see Fig. 1 for the map positions of the fragments). The input radioactivity of the RNA used for the hybridization was about 4,000 cpm.

[b] Letters in parentheses indicate genes.

[c] Background value (no DNA in hybridization mixture) was subtracted.

[d] Numbers in parentheses are percentages. The hybridization values for wild-type cells and *nitA* cells infected with wild-type phage were defined as 100%.

Polarity was not caused by premature termination of transcription at the site of the polar mutation. Table 3 indicates that wild-type cells infected with a polar gene F mutant (H244) contained reduced amounts of gene G and H messages but synthesized a considerable amount of gene F message. This implies that RNA synthesis in these cells continued to the end of gene F (T_F). Figure 3b shows that peak V and VI RNAs contained the message for gene F but were not eliminated by the polar mutant H244 in wild-type cells. The RNAs from peaks V and VI of H244-infected cells were identical in cistron content to peak V and VI RNAs from cells infected with wild-type phage. All of these RNAs hybridized to restricted DNA fragments corresponding to the carboxyl-terminal end of gene F. Neither peak VI RNA from cells infected with wild-type phage nor peak VI RNA from phage H244-infected cells hybridized to restricted DNA fragments corresponding to the A:B region of the genome (Table 4). Thus, peak V RNA and peak VI RNA from H244 infections of wild-type cells were not truncation products of peak II, IV, or V RNA arising from premature termination at or near the site of the H244 mutation. The same situation seemed to exist for peak IV RNA from am9-infected wild-type cells. Table 3 shows that wild-type cells infected with am9 (a polar G mutant) synthesized a considerable amount of gene G message compared with wild-type cells infected with wild-type phage. The RNA in peak IV contained the site of the am9 mutation but apparently terminated at T_G since its electrophoretic mobility was not affected (Fig. 4b).

***nitA* mutation relieves polarity caused by chloramphenicol.** Transcription of certain bacterial operons and transcription of portions of the T4 phage genome are prevented by treating the host cells with the protein synthesis inhibitor chloramphenicol. That this reflects a form of polarity is shown by the fact that tran-

FIG. 4. *Size distribution of φX174 mRNA's in cells infected with a polar gene G mutant. This experiment was identical to the experiment described in the legend to Fig. 3, except that nitA cells (a) or wild-type cells (b) were infected with am9 phage (gene G polar mutant). S·S DNA, Single-stranded DNA derived from mature phage and replicative intermediates of DNA.*

TABLE 4. *Relative number of sequence copies of each RNA size class*

Phage	RNA peak	Relative no. of sequence copies of each RNA size class[a]							
		R4	R3	R8	R5	R7b	*Hap*II-*Hpa*I	R1	R2
Wild type	V	0.30	0.18	0.94[b]	1.25[b]	1.05[b]	1.25[b]	1.17[b]	0.21
	VI	0.33	0.23	0.30	0.33	0.51	2.22[b]	2.06[b]	0.39
	IX	0.18	0.16	1.74[b]	2.18[b]	2.12[b]	2.16[b]	0.17	0.13
	XIII	0.22	0.19	0.27	0.17	0.51	3.00[b]	0.14	0.17
H244	V	0.37	0.32	1.25[b]	1.87[b]	1.69[b]	1.67[b]	1.75[b]	0.49
	VI	0.37	0.32	0.22	0.34	0.18	1.67[b]	2.12[b]	0.35
	IX	0.22	0.34	1.74[b]	1.54[b]	1.88[b]	1.99[b]	0.21	0.22
	XIII	0.29	0.31	0.29	0.21	0.21	2.23[b]	0.39	0.14

[a] Each RNA size class was eluted from gels and hybridized to restriction fragments of RF DNA as described in a previous paper (10). The input radioactivity of the RNA used for the hybridization was about 2,500 cpm. The relative numbers of sequence copies were calculated from the following expression: [(counts hybridized to fragment)/(total input counts)] × [(size of total genome)/(size of fragment)]. R1 through R5, R7b, and R8 were fragments with *Hin*dIII. For *Hap*II-*Hpa*I, the largest fragment with *Hap*II was digested with *Hpa*I, and the second largest fragment was used (*Hap*II-1-*Hpa*I-2). The orientations of these fragments with respect to the total genome are shown in Fig. 1.

[b] RNA copy contains the fragment region.

scription is restored if a rho mutation is introduced into the cells (1, 22, 23).

Similar results were obtained when φX174-infected cells were treated with chloramphenicol. When wild-type phage-infected wild-type cells were pulse-labeled with [^{3}H]uridine in the presence of this drug, the size distribution of φX174 mRNA was different from the size distribution in untreated cells. The predominant RNA products in the presence of the drug were peak IX RNA and peak XIII RNA. However, RNA larger than peak IX RNA was synthesized when infected *nitA* cells were treated with chloramphenicol (Fig. 5a and b). The amounts of peak IX RNA and peak XIII RNA in Fig. 5a and b are almost identical, but Fig. 5a shows that *nitA* cells contained considerable counts in RNAs larger than peak IX RNA. When the total φX174 mRNA's isolated from *nitA* or wild-type cells infected with wild-type phage in the presence of chloramphenicol were hybridized to restriction fragments, *nitA* cells contained three to

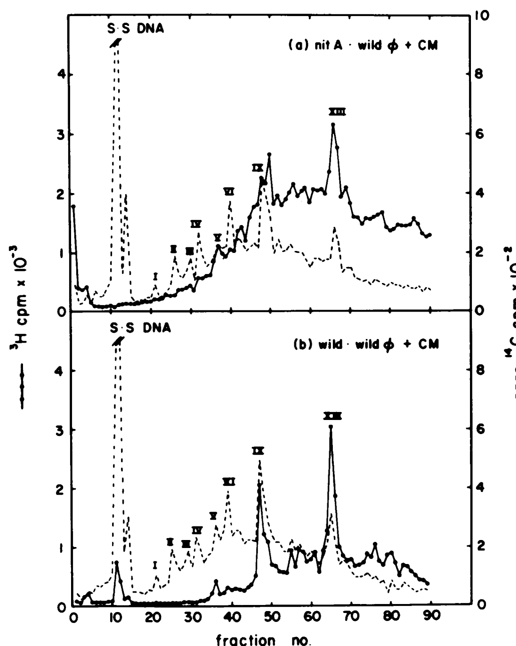

FIG. 5. *Effects of chloramphenicol on size distribution of RNAs synthesized in nitA cells and wild-type cells. nitA (a) or wild-type (b) cells were infected with wild-type phage. Chloramphenicol (100 μg/ml) was added to the infected cultures 9 min after infection; 2 min after chloramphenicol was added, [^{3}H]-uridine was added to the cultures. Incubation was continued for an additional 5 min. φX174 mRNA's were isolated and analyzed by using agarose-urea gels. [^{14}C]RNA was prepared as described in the legend to Fig. 1. S·S DNA, Single-stranded DNA derived from mature phage and replicative intermediates of DNA.*

five times more gene F, G, and H messages than were present in wild-type cells (data not shown).

DISCUSSION

φX174 transcription termination sites in *nitA* cells are similar to φX174 transcription termination sites in wild-type cells. Figures 2a and b show that the RNA species present in wild-type cells are found in cells deficient in rho activity. With or without rho activity, transcription termination at sites T_J, T_F, T_G, and T_H was observed. No preferential synthesis of larger RNAs was found in *nitA* cells. The simplest interpretation of these results is that normal termination sites function in *nitA* cells. The alternative explanation of residual rho activity at the restrictive temperature does not seem likely since rho-mediated polarity is relieved effectively under these conditions (Fig. 3 through 5; see below). However, higher background counts (φX174 specific) distributed throughout the gel patterns of RNAs from *nitA* cells may indicate that in the absence of rho activity, transcription termination is disturbed so that more "randomly" terminating RNA species are produced. Alternatively, it is also possible that the production of this "background" RNA is inherent in *nitA* mutants due to pleiotropic effects (see below) other than involvement in transcription termination (for example, mRNA degradation).

Axelrod (2, 3), Smith and Sinsheimer (30–32), and Kapitza et al. (18, 19) determined the in vitro termination sites of φX174 transcription. Some sites are rho independent, and some are not. The results of these authors are summarized in Fig. 1. Aside from the correspondence of the in vitro termination sites T_2 (rho independent) and T_4 (rho independent) of Axelrod with the in vivo terminators T_J and T_H, respectively, and the correspondence of the in vitro termination site T_3 (rho dependent) of Kapitza et al. with the in vivo terminator T_F, results from in vivo and in vitro studies on the termination sites do not agree. This may indicate that the participation of cellular components other than RNA polymerase and rho factor (if needed) are required for in vivo termination.

Gilbert (9) proposed that termination of transcription occurs by dissociation of the transcriptional complex at sites on the DNA at which RNA polymerase pauses during the elongation process (20). He suggested that the probability of termination at any particular site is determined by the duplex DNA stability at that site. McMahon and Tinoco (21) analyzed known termination sequences in terms of duplex stability arising from nearest-neighbor interactions and found that these sequences exhibited a charac-

teristic pattern of nearest-neighbor stabilities. They then looked for regions having this pattern of nearest-neighbor interactions in the sequence of φX174 DNA proposed by Sanger et al. (26) and found only five such sites. Interestingly, these five theoretically possible termination sequences seem to coincide with the termination sites T_1, T_J (T_2), F_F, T_G, and T_H (T_4) suggested by in vivo and in vitro results (Fig. 1).

***nitA* mutation relieves polarity.** Although normal termination sites of φX174 in vivo seem to be recognized in *nitA* cells, rho activity is responsible for the polar effects caused by nonsense mutations in the phage genome or by chloramphenicol treatment of the host cells. The introduction of the *nitA* mutation in these situations relieves polarity. This effect is strong in the case of polarity caused by nonsense mutations (Fig. 3 and 4) and is partial in chloramphenicol-treated cells (Fig. 5).

Adhya and Gottesman have proposed a model to explain the involvement of rho in polarity (1). The nascent mRNA is normally covered with ribosomes, which prevent the action of rho factor. When ribosomes are released from mRNA (usually some distance beyond the chain-terminating nonsense codon), rho is able to bind to and move down the RNA chain until it reaches the transcription complex paused at a transcriptional termination site. Rho interacts with the polymerase molecule, resulting in the release and, thus, termination of the mRNA. However, if the nonsense codon (either the natural chain-terminating codon or a codon resulting from a mutation) is followed by a restart codon, translation resumes and rho activity is abolished. Such mutations are nonpolar.

When a nonsense mutation is introduced near the amino terminus of gene F (H244), translation is terminated at the mutation site. Apparently, RNA synthesis continues until the first termination site which coincides with T_F is reached. Rho then causes termination at this site, resulting in the formation of peak V RNA and peak VI RNA. The nonsense mutation at am9 (near the N terminus of gene G) stops translation, and rho functions when the RNA polymerase reaches the nearest termination signal near or at T_G. The action of rho at this site results in the detection of peak IV RNA and the elimination of peak II RNA. We would expect a new peak larger than peak VI RNA and smaller than peak V RNA (promoter D; terminator T_G). This peak has not been detected in cells infected with wild-type phage because of the limited resolution of the gel system. Similarly, chloramphenicol freezes ribosome movement along mRNA, thus creating unprotected regions of mRNA which are susceptible to rho action (22, 35). As Fig. 5b shows RNAs synthesized in the presence of chloramphenicol start at promoters B and D and are all terminated at the first termination signal near or at T_J by the action of rho. Therefore, peak IX RNA and peak XIII RNA are the main transcripts in wild-type cells. However, in the absence of rho (Fig. 5), termination at T_J is not absolutely stringent, and a fraction of RNA polymerase transcribes beyond this site.

These results indicate that sites T_J, T_F, and T_G are rho dependent when ribosomes are absent around these sites and rho independent when active protein synthesis takes place. Apparently, in the absence of rho factor the transcriptional (and possibly translational) machinery does not contain a termination mechanism as stringent as rho factor. Whether the natural terminators T_J, T_F, and T_G are exactly identical to termination sites caused by polarity must be determined by sequence data for these sites.

Pleiotropic effects of the *nitA* mutation on φX174 development. Although termination of φX174 transcription in vivo seems to occur normally in *nitA* cells, phage development is inhibited in *nitA* cells. When infected *nitA* and wild-type cells are compared, several differences are recognized. (i) The rates of host and φX174-specific RNA synthesis are reduced in *nitA* cells. (ii) The background RNA (see above) in gel profiles of infected *nitA* cells increases. (iii) Although the parental single-stranded DNA is converted to RF DNA normally in *nitA* cells, subsequent RF DNA replication and single-stranded DNA synthesis are reduced (manuscript in preparation). It is possible that φX174 development requires the continuous, de novo synthesis of a host-originated factor(s) which is inhibited when rho factor is inactivated. Alternatively, or in addition, rho may have additional functions besides transcription termination. For example, rho may be involved in the initiation of transcription, elongation of transcripts, mRNA metabolism and DNA replication.

Pleiotropic phenotypes of rho mutants have been reported. These include hyperdegradation of protein (29) deficiencies in membrane ATPase (6), deficiences in recombination and UV repair (5), and deficiencies in the ability to support the growth of certain phages (5, 16). Adhya and Gottesman (1) have suggested that these defects appear to involve the loss of ATPase activity and that the products of the rho gene are involved in a variety of cellular reactions which utilize ATP. Further investigations will be required to decide whether the pleiotropic effects of the *nitA* mutant described in this paper are explained by this suggestion.

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