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Interaction of RNA polymerase and rho in transcription termination: Coupled ATPase

(suppressor RNA polymerase/IS2 element/*gal* operon/*rho* alleles/phage λ)

ASIS DAS*†, CARL MERRIL‡, AND SANKAR ADHYA*

* National Cancer Institute and ‡ National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20014

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ABSTRACT **We have previously described temperature sensitive *rho* mutants of *Escherichia coli* (e.g., *rho15*) that are defective in transcription termination at various signals, including an IS2 DNA insertion in the *gal* operon [Das, A., Court, D. & Adhya, S. (1976) *Proc. Natl. Acad. Sci. USA*, 73, 1959–1963]. In this paper, we report the isolation of mutants altered in the β subunit of RNA polymerase (a class of Rifampicin-resistant mutants), which restore *gal* IS2 polarity in the *rho15* strain. It has been shown that one of these suppressor RNA polymerases (*rpoB101*) requires rho to terminate transcription of phage λ mRNA. In contrast to the wild type RNA polymerase, the suppressor RNA polymerase also terminates λ mRNA transcription in the presence of rho15 protein. We have isolated new *rho* mutants (e.g., *rho112*) that are defective in transcription termination in the *rpoB101* strain. These results strongly support the notion that rho and RNA polymerase interact functionally during transcription termination.**

We have shown that rho15 catalyzes ATP hydrolysis during transcription with *rpoB101* RNA polymerase, but not with wild-type RNA polymerase. Because rho15 protein hydrolyzes ATP in the presence of free RNA, we suggest that rho may recognize the 3′-OH end of RNA. During transcription, this recognition involves an interaction with RNA polymerase, resulting in the displacement of the polymerase and the release of the nascent mRNA.

Rho protein of *Escherichia coli* provokes termination of transcription at sites located at the ends of or within operons (1, 2). A mechanism of rho-mediated transcription termination has been recently proposed (reviewed in ref. 3). Briefly, the termination of mRNA synthesis *in vivo* is triggered by the dissociation of ribosomes from nascent mRNA at a nonsense codon. Rho attaches to the untranslated mRNA and migrates toward an RNA polymerase molecule paused at the transcription termination site. The RNA-bound rho and RNA polymerase then interact to effect the termination and release of RNA chains. Transcription termination by rho requires ATP hydrolysis (4, 5). Although it appears that rho and RNA polymerase may form a complex (6, 7), the function of such a complex in transcription termination has not been demonstrated. The analysis of transcription termination has been facilitated by the isolation of mutants defective in rho protein (8–12). Outside suppressors of the rho phenotype have been isolated as rifampicin-resistant mutants; these *rpoB* mutants have an altered β subunit of RNA polymerase (13, 14). We report here a genetic and biochemical analysis of an RNA polymerase mutation (*rpoB101*), which suppresses *rho15* and restores transcription termination in this strain. The *rpoB101* RNA polymerase terminates transcription in the presence of rho15 protein. New *rho* mutants have been isolated that are not suppressed by *rpoB101* RNA polymerase. These data provide evidence for a functional interaction between rho and RNA polymerase during transcription termination.

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MATERIALS AND METHODS

Isolation of *rpoB101* RNA Polymerase Mutant. Spontaneous Rifampicin-resistant (at 50 μg/ml) derivatives of *E. coli* strain AD1600 (F⁻ *galP-E3::IS2 his rho15 str*^{R}) were isolated at 30° on tryptone/agar plates at a frequency of approximately 10^{-9}. Individual colonies were tested for their Gal phenotype on McConkey galactose/agar plates at 30°. Twenty-four Gal⁻ derivatives were detected among 220 colonies. The possibility that the Gal⁻ phenotype of these strains was due to a second mutation rather than to the Rif^R mutation, was tested as follows: (*i*). *Gal⁺ reversion.* Each Rif^R Gal⁻ strain was compared with the *rho⁺ Rif*^{S} parent strain for Gal⁺ reversion frequency. A *gal* mutation in addition to *galP-E3* would be expected to reduce this frequency. (*ii*). *Recombination with* λgal8-165. This transducing phage carries a deletion encompassing most of *galK* and *galE* and all of *galT* (15). The phage yields *gal⁺* transductants with the *galP-E3* strain, showing that it carries an intact *galOP* region. Production of *gal⁺* transductants on tryptone/triphenyltetrazolium chloride/galactose plates with various Rif^R Gal⁻ strains is consistent with the absence of a second *gal* mutation in the strains. (*iii*). *Pleiotropic properties of rho15.* To determine if the Rif^R Gal⁻ strains retained the *rho15* mutation, we checked other properties characteristic of this mutation (e.g., *ts*, Suc⁻, resistance to phage P2, and UV sensitivity).

On the basis of the above tests, four strains (*rpoB101–104*) appeared likely to be RNA polymerase mutants that restored transcription termination in the *rho15* mutant strain but did not alter the remaining phenotype of *rho15* mutation.

Isolation of the *rho112* Mutant. The *rho112* mutant, among several others, was isolated as described (9) as a Gal⁺, temperature-sensitive derivative of strain AD1772 (F⁻*galP-E3 his⁻str*^{R}*rpoB101 val*^{R}). Phage P1 grown on AD1772 *rho112* was used to transduce either strain SA1030 (F⁻*galP-E3 his*), thereby selecting for Val^R, or an *ilv* derivative of SA1030, thereby selecting for Ilv⁺, on minimal glucose/agar plates at 30°. The *val*R or *ilv⁺*, transductants were tested for temperature sensitivity and Gal⁺.

Purification of Rho. Rho protein was purified by using the procedure of Roberts (1), with some modifications, from strains SA500 F⁻*his⁻Str*^{R} (*rho⁺*), AD1600 (*rho15*), and AD1919 (*rho112*) grown to OD_{650} = 1.0 at 30° in LB-glucose medium.

After the phosphocellulose step, pooled rho fractions (determined by poly(C)-dependent ATPase activity at 32°) were

† Present address: Laboratory of Tumor Virus Genetics, National Cancer Institute, Bldg. 41, National Institutes of Health, Bethesda, MD 20014.

concentrated by ammonium sulfate precipitation (65% saturation), dialyzed against 10 mM K phosphate, pH 7.5/0.1 mM dithiothreitol/10% glycerol, and chromatographed on an SP-Sephadex C25 column previously equilibrated with 10 mM K phosphate buffer (pH 7.5) (L. Finger and J. Richardson, personal communication). Rho was eluted from the column by a linear gradient of 10–500 mM K phosphate; rho elutes at approximately 45 mM K phosphate. Active fractions were pooled, diluted to 30 mM K phosphate and passed through a small phosphocellulose column. Rho was eluted with 0.5 M K phosphate buffer, pH 7.5/0.2 mM dithiothreitol/20% glycerol.

Purification of RNA Polymerase. RNA polymerase from strains SA500 (*rpoB*$^+$) and AD1772 (*rpoB101*) was purified by using the procedure of Burgess (16) with the following modifications. After the DEAE cellulose step, pooled fractions active in RNA synthesis on phage λ DNA template were precipitated with 1.5 volumes of saturated ammonium sulfate. The precipitate was then dissolved in buffer (50 mM Tris·HCl, pH 7.9/1 M NaCl/10 mM $MgCl_2$/0.1 mM EDTA/0.1 mM dithiothreitol/10% glycerol) and passed through a Sepharose 4B column (previously equilibrated with the same buffer). Active fractions were pooled and stored in the storage buffer described by Burgess (16) at −70°. RNA polymerase purified in this way was essentially free of nucleases, because it produces discrete 10–12S, 8–9S, 6S, and 4S λ mRNA transcripts in the presence of rho (17).

Assay of ATPase Activity of Rho. Poly(C)-dependent ATPase activity of rho was measured in 100-μl reaction mixtures containing 40 mM Tris·HCl (pH 7.9), 50 mM KCl, 12 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.7 mg of bovine serum albumin per ml, 10 μg of poly(C) per ml, 0.4 mM ATP, and 0.005 μCi of [γ-^{32}P]ATP per μl. The reaction was initiated by the addition of rho (1–2 μg) and, after incubation at 32° for 15 min, the reaction mixture was chilled in ice. Inorganic $^{32}PO_4$ was measured according to published procedures (18): to the mixture is added 0.1 ml of 20 mM silicotungstic acid in 10 mM H_2SO_4, 0.2 ml of 2 mM K phosphate (pH 6.0), and 0.1 ml of 5% ammonium molybdate in 4 M H_2SO_4. The mixture was incubated at 37° for 2 min, and 0.5 ml of benzene/isobutanol (1:1) was added. The suspension was Vortex mixed and $^{32}PO_4$ was extracted in the solvent layer. Radioactivity in 0.1 ml of the top layer was measured in 5 ml of Aquasol.

[γ-^{32}P]ATP was obtained from Amersham/Searle and [α-^{32}P]ATP, from New England Nuclear. [^{14}C]galactose was purchased from Amersham/Searle. SP-Sephadex C25 was purchased from Pharmacia. ATP, GTP, CTP, UTP, and poly(C) were from P-L Biochemicals.

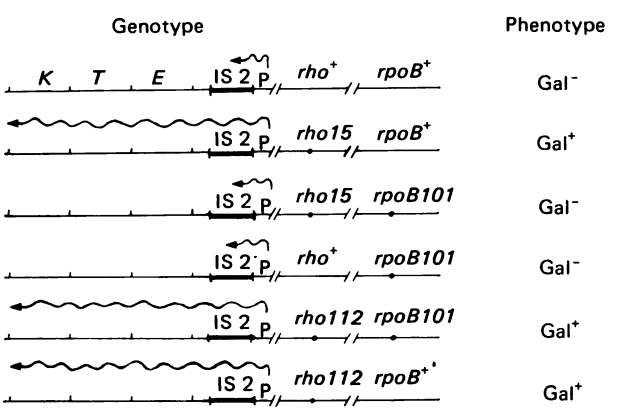

FIG. 1. Relevant genotypes and derivations of strains of *E. coli* K-12 used.

RESULTS

The relevent genetic structures of the *E. coli* K-12 strains used in this study and the derivation of these strains are shown schematically in Fig. 1. All strains carry the IS2 insertion mutation, *galP-E3*, located in the leader region of the *gal* operon. The insertion is severely polar on the expression of the three *gal* cistrons, *K*, *T*, and *E*. The polarity results from rho-mediated transcription termination within the IS2 insertion (Fig. 1, line 1); selection for Gal$^+$ revertants has yielded *rho* mutations, such as the temperature sensitive *rho15* (9). Our *in vitro* experiments support the idea that the *rho15* strain is Gal$^+$ because the rho15 protein cannot provoke transcription termination at rho-dependent sites. Thus rho15 *in vitro* fails to catalyze the termination of λ mRNA synthesis or to hydrolyze ATP during transcription at any temperature, although it retains a temperature sensitive, poly(C)-dependent ATPase activity (9). We show below that the defect of rho15 in transcription termination, both *in vivo* and *in vitro*, can be corrected by an alteration in RNA polymerase.

Suppressor Mutation in the β Subunit Gene (*rpoB*) of RNA Polymerase. Mutations conferring resistance to rifampicin alter the β subunit of RNA polymerase (19). Spontaneous rifampicin-resistant (Rif^R) derivatives of the *rho15* mutant were isolated and then screened for their Gal phenotype (see *Materials and Methods*). Twenty-four of 220 Rif^R derivatives thus isolated scored as Gal$^-$. Twenty of these Rif^R Gal$^-$ mutants appeared either to have a second mutation in the *gal* operon or to be revertants of the *rho15* mutation. In the remaining four, the RNA polymerase mutation (*rpoB101-104*) permits tran-

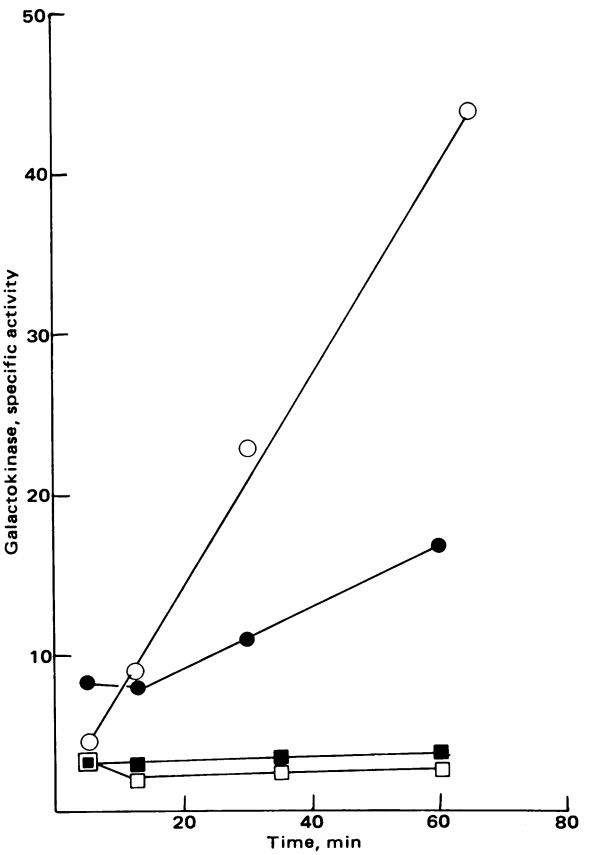

FIG. 2. Galactokinase induction from *galE-P3* in various mutant strains. Cells were induced for *gal* enzymes by D(+)-fucose addition at 0 min. Growth of cells in minimal media and the galactokinase assay were performed as described (9). ○, *gal*$^+$ *rho*$^+$; ●, *gal3 rho15*; □, *gal3 rho*$^+$; ■, *gal3 rho15 rpoB101*.

scription termination within IS2 in the *rho15* strain. This conclusion is based on the following results.

A strain carrying both *rho15* and *rpoB101* mutations was reconstructed. First, phage P1 was used to transduce the *rpoB101* marker into a *rho*$^+$ *galP-E3* strain by selecting for Rif^R (Fig. 1, line 4). Like its *rpoB*$^+$ parent, the transductant is Gal$^-$. The *rho15* mutation was then introduced into this strain by using phage P1 grown on a *val*^{R} *rho15* host. The *val*^{R} marker maps in the *ilv* locus, closely linked to *rho*. Out of 24 Val^R transductants, 19 were temperature sensitive for growth, UV sensitive, resistant to phage P2, and Suc$^-$; these phenotypes are characteristic of a *rho15* mutant strain (20). However, like their *rho*$^+$ parent (Fig. 1, lines 3 and 4), all 24 Val^R transductants remained Gal$^-$. We assayed the level of galactokinase, the *galK* product, in one of the *ts* transductants (*rho15 rpoB101*). Consistent with the Gal$^-$ phenotype, the kinase activity was very low (Fig. 2). These results indicate that the *rpoB101* mutation has altered the RNA polymerase to restore transcription termination within IS2 in the *rho15* strain. We conclude that the *rpoB101* or a closely linked mutation is a suppressor of the *rho15* allele.

The *rpoB101* RNA polymerase may restore transcription termination in the *rho15* mutant in two ways: (*i*). The mutant RNA polymerase terminates transcription at rho-sensitive signals in the absence of rho. This was suggested by Guarente and Beckwith (13) from their studies with a similar mutation, *rpoB203*, which restores transcription termination at the end of the *trp* operon in several mutant *rho* strains, including a *rho*am(*nit112*) mutant (11). (*ii*). The *rpoB101* RNA polymerase interacts with the rho15 protein to terminate transcription. The two models lead to very different genetic and biochemical predictions. In the first model, *rpoB101* RNA polymerase will not require rho to terminate mRNA synthesis *in vitro*. In addition, no mutation in *rho* will affect transcription termination in the *rpoB101* strain. In the second model, the mutant RNA polymerase will still require rho to terminate mRNA synthesis and will terminate transcription with wild-type rho or rho15. Furthermore, it might be possible to isolate a *rho* mutant strain that is not suppressed by the *rpoB101* mutation.

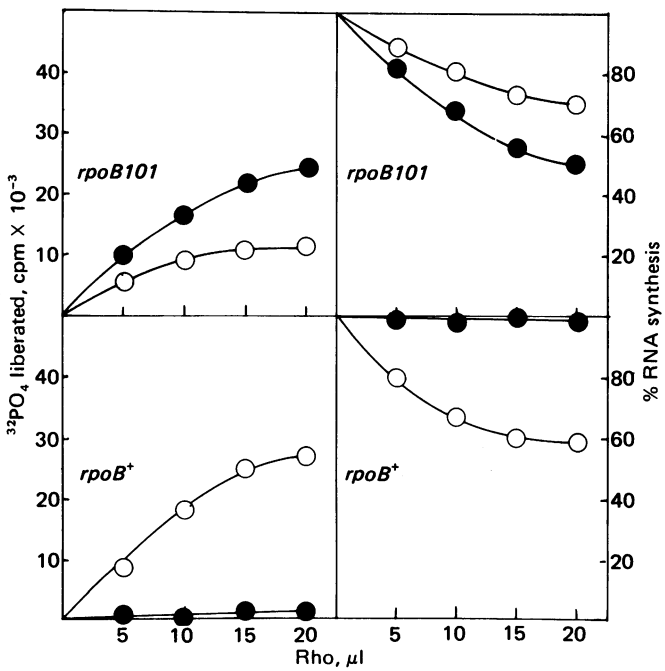

FIG. 3. Transcription termination by rho15 with *rpoB101* RNA polymerase. Rho proteins from strains SA500 (*rho*$^+$) and AD1600 (*rho15*) and RNA polymerases from strains SA500 (*rpoB*$^+$) and AD1772 (*rpoB101*) were purified as described in *Materials and Methods*. Transcription termination activities of the two rho proteins were measured by the reduction of mRNA synthesized by the two RNA polymerases in 100 μl of the following reaction mixture: 40 mM Tris·HCl, pH 8.0/50 mM KCl/12 mM $MgCl_2$/0.1 mM EDTA/0.1 mM dithiothreitol/0.3 mg of bovine serum albumin per ml/0.4 mM each of ATP, GTP, CTP, and UTP/0.01 μCi of [α-^{32}P]ATP per μl/5 μg of DNA. Wild-type (11 μg) and *rpoB101* (14 μg) RNA polymerases were used. Concentrations of the wild-type and the mutant rho preparations used were 0.15 mg/ml and 0.18 mg/ml, respectively. Reactions were initiated by transferring the incubation mixtures from 0° to 30°. After 15 min, trichloroacetic acid insoluble radioactivity was measured. Nascent RNA-dependent ATPase activities of rho were measured in the reaction mixtures described above, but with [γ-^{32}P]ATP (0.02 μCi/μl) instead of [α-^{32}P]ATP. A background of 7000 cpm and 5500 cpm was obtained for the wild-type and the mutant RNA polymerases alone, respectively, and was subtracted from values obtained in the presence of rho. O, Rho$^+$; ●, rho15.

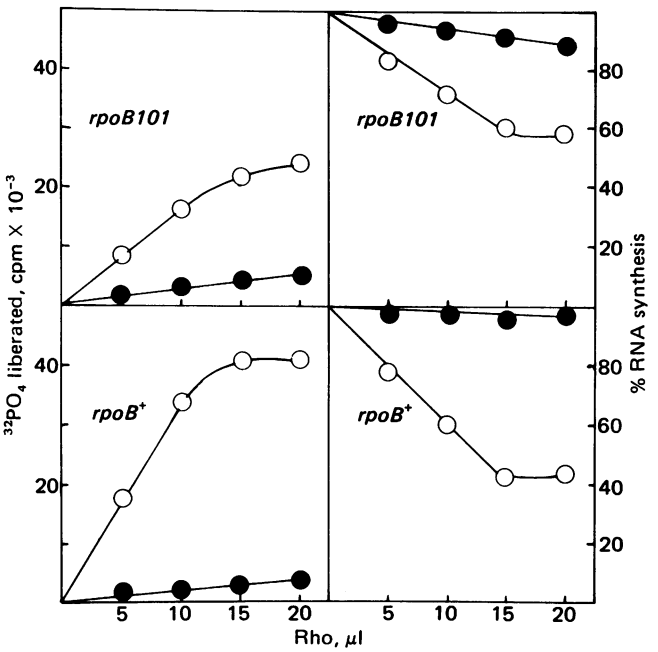

FIG. 4. Temperature sensitivity of the transcription termination activity of rho15. The reactions were carried out as described in Fig. 3, except that the incubations were performed at 38°. In the absence of rho, a background of 8800 and 6400 cpm in $^{32}PO_4$ was obtained with the wild-type and the mutant RNA polymerase, respectively, and was subtracted from the values obtained in the presence of rho. O, Wild-type rho; ●, rho15.

Rho15 Protein Terminates λ mRNA Transcription by *rpoB101* RNA Polymerase. Termination of phage λ mRNA transcription was studied by using wild-type and mutant RNA polymerases. Previous studies with *rpoB*+ RNA polymerase showed that discrete λ mRNA species 10–12S (*N* RNA) and 8–9S (*cro* RNA) are produced in the presence of wild-type rho (1, 17). An identical pattern of transcription was observed with the *rpoB101* RNA polymerase. In the presence of wild-type rho, bands corresponding to 10–12S and 8–9S RNA were observed in urea/polyacrylamide gels; in its absence no such bands were observed (data not shown). We next compared the abilities of wild-type rho and rho15 to produce λ mRNA termination by measuring the net decrease in total RNA synthesis at various concentrations of the termination factors (1). As shown in Fig. 3 *right*, at 30°, the addition of increasing concentrations of wild-type rho decreased RNA synthesis by up to 40% with *rpoB*$^+$ RNA polymerase and by up to 30% with *rpoB101* RNA polymerase. Rho15, on the other hand, decreased RNA synthesis by up to 50% with *rpoB101* RNA polymerase but by less than 5% with *rpoB*$^+$ RNA polymerase.

Table 1. Behavior of rho proteins

	Rho+	Rho15	Rho112
Molarity of K phosphate that elutes rho from PC	0.16	0.12	0.14
Relative sedimentation through glycerol gradient	Tetramer*	Dimer	Dimer
Apparent M_r†	54,000	~52,000	~50,000
Relative yield‡	1	~2	~5

PC = phosphocellulose column.

* Wild-type rho has been shown to sediment as a tetramer (1).

† The apparent molecular weights were determined by comparing mobilities in sodium dodecyl sulfate/gel electrophoresis with known markers.

‡ Approximate relative yields, assuming equal loss during purification, from same amount of cells. Activities were determined by poly(C)-dependent ATP hydrolysis at 30°. Rho protein is often produced in excess by *rho* mutant strains (7).

The presence of rho in a transcription reaction leads to the extensive hydrolysis of ATP (21); the hydrolysis of ATP is essential for termination (4, 5). Rho15 is defective in this reaction at all temperatures tested (9). When *rpoB101* RNA polymerase was used to transcribe λ DNA, however, the ATPase activity of rho15 was restored (Fig. 3, *left*). These results demonstrate that the termination and ATPase activities of rho15 can be restored by an alteration in RNA polymerase.

Both termination activity and the ATPase activity of rho15 during transcription are temperature sensitive. At 38°, in contrast to the wild-type rho protein, rho15 fails to display either activity (Fig. 4). The two activities of rho, therefore, cannot be uncoupled.

A Rho Mutant in which *rpoB101* RNA Polymerase Fails to Terminate Transcription. To demonstrate that the suppression of a *rho* mutation by *rpoB101* is allele specific, we isolated temperature-sensitive rho mutants starting with the *rho+ rpoB101 galP-E3* strain (Fig. 1, line 4). Gal+ revertants were isolated at 32° (see *Materials and Methods*). About 70% of these revertants failed to grow at 42°. Four *ts* mutations (*rho105, rho108, rho109,* and *rho112*) were transferred into the *galP-E3* strain (Fig. 1, line 1) by P1 transduction selecting for Val^R (see *Materials and Methods*). About 70–80% of the Val^R transductants were temperature sensitive. The *ts* transductants were Gal+ and in addition, UV sensitive, Suc−, and, except for *ts112*, resistant to phage P2. These results strongly suggest that the *ts* mutations are located in the *rho* locus. Accordingly, we isolated and analyzed the rho protein from the *rho112* mutant.

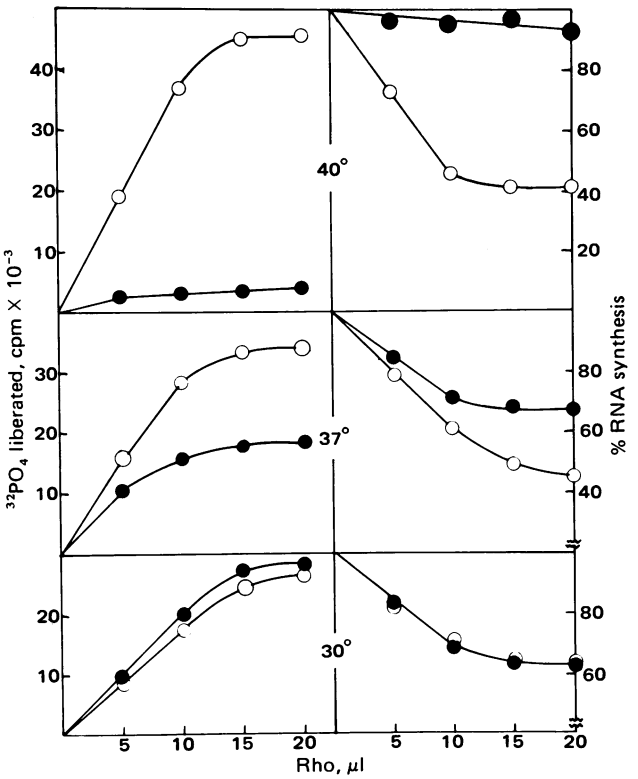

FIG. 6. Transcription termination activity and the nascent RNA dependent ATPase activity of rho112. The reactions were carried out as described in Figs. 3 and 4. The concentration of rho112 protein in the preparation used was 210 μg/ml. In the absence of rho, backgrounds of 7300, 8500, and 9000 cpm in $^{32}PO_4$ were obtained at 30°, 37°, and 40°, respectively, and were subtracted from values obtained in the presence of rho. O, Wild-type rho; ●, rho112.

Physical and Functional Properties of Rho15, Rho112, and Wild-Type Rho. Rho112 was purified as described in *Materials and Methods* and compared with wild-type rho and rho15. As summarized in Table 1, the three proteins have different physical properties. They elute with different concentrations of phosphate buffer from the phosphocellulose column. Rho15 and rho112 sediment as dimers in glycerol gradients; wild-type rho sediments as a tetramer. When subjected to electrophoresis in sodium dodecyl sulfate/polyacrylamide gels, both rho15 and rho112 migrate faster than wild-type rho; the three proteins

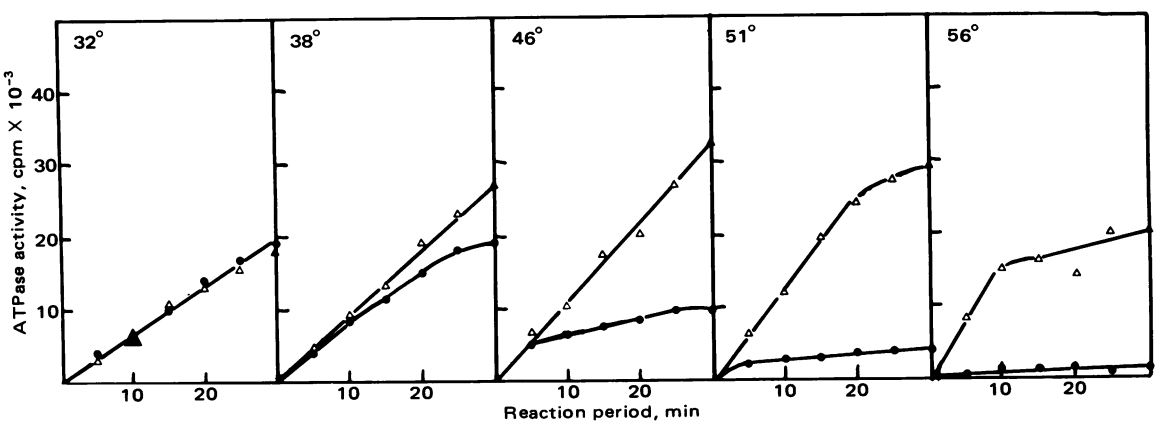

FIG. 5. Poly(C)-dependent ATPase activity of rho112 at different temperatures. The determination of the ATPase activity of rho112 and rho in the presence of poly(C) at various temperatures was as described in *Materials and Methods*. In each case, the reaction was initiated by the addition of rho112 or rho to 1 ml of a reaction mixture prewarmed at the indicated temperatures. Samples (50 μl) were withdrawn at various intervals for the determination of $^{32}PO_4$. Δ, Wild-type rho; ●, rho112.

migrate with apparent molecular weights of 52, 50, and 54 kilodaltons, respectively.

The poly(C)-dependent ATPase activity of rho112 was compared with that of wild-type rho at various temperatures. The ATPase activity of both rho15 and rho112 was temperature sensitive (9; see Fig. 5). Rho112 shows a temperature optimum for this reaction at 38°, and very little activity at 51°, which is the optimal temperature for wild-type rho (Fig. 5). No apparent difference in the temperature sensitivity for the poly(C)-dependent ATPase activity between the two mutant rho proteins was observed. Both are irreversibly inactivated at 50° (data not shown).

A functional difference between rho112 and rho15 was observed when transcription termination and ATP hydrolysis were measured at different temperatures. At 30° with *rpoB*$^+$ RNA polymerase, rho112 displayed both activities (Fig. 6). Rho112 retained some activity at 37°, but failed to cause either transcription termination or ATP hydrolysis at 40°. Recall that rho15 was inactive in these reactions at any temperature. For reasons as yet not understood, rho112 showed considerable ATPase activity and transcription termination with *rpoB101* RNA polymerase, although both activities were reduced compared to *rpoB*$^+$ RNA polymerase.

DISCUSSION

An IS2 DNA insertion element carrying a rho-sensitive transcription termination signal is nonpolar in the *rho15* mutant (22). We have succeeded in isolating suppressor mutations, located in the cistron encoding the β subunit of RNA polymerase, that restore IS2 polarity in the *rho15* strain. One such suppressor, *rpoB101*, is clearly allele specific; it does not restore the polarity of an IS2 insertion in another *rho* mutant strain, *rho112*. The *rpoB101* RNA polymerase does not, by itself, terminate transcription at rho-sensitive signals on λ DNA template. However, the *rpoB101* RNA polymerase can, unlike *rpoB*$^+$ RNA polymerase, terminate transcription at rho-sensitive signals in the presence of rho15 and may, in fact, be more responsive to the mutant than the wild-type rho. These genetic and biochemical data clearly establish a functional interaction between rho and RNA polymerase during transcription termination. Our results suggest the possibility of isolating RNA polymerase mutants defective in transcription termination in the *rho*$^+$ strains. Some of the RNA polymerase mutants (*nitB*) isolated by Inoko and Imai (23), may be of this type.

Rho protein shows two ATPase activities: ATPase activity observed in the presence of free RNA (uncoupled ATPase) and ATPase activity associated with transcription termination (coupled ATPase). Rho15 displays a temperature-sensitive uncoupled ATPase activity but fails to show termination of transcription and coupled ATPase activity at any temperature with wild type RNA polymerase. However, at 30° both the termination activity and the coupled ATPase activity of rho15 are restored when *rpoB101* RNA polymerase replaces *RpoB*$^+$ RNA polymerase. One evident difference between the RNA molecule in the coupled and the uncoupled ATPase reactions is the availability of the 3′-OH end. We propose that both ATPase activities of rho are associated with its contact with the 3′-OH end of RNA. In the coupled system rho interacts with and displaces RNA polymerase from the 3′-OH end of the RNA, causing the release of the transcript from the transcription complex. ATP hydrolysis accompanies this displacement reaction and is essential for transcription termination. After transcription termination, rho, in conjunction with the released transcript, continues to catalyze ATP hydrolysis. We assume that this uncoupled ATPase is not related to transcription termination, because this activity is displayed by rho15, which is normally inactive in transcription termination.

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1. Roberts, J. (1969) *Nature (London)* **224,** 1168–1174.
2. deCrombrugghe, B., Adhya, S., Gottesman, M. & Pastan, I. (1973) *Nature (London) New Biol.* **241,** 260–264.
3. Adhya, S. & Gottesman, M. (1978) *Annu. Rev. of Biochem.* **47,** 967–996.
4. Galluppi, G., Lowry, C. & Richardson, J. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M., (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 657–665.
5. Howard, B. & deCrombrugghe, B. (1976) *J. Biol. Chem.* **251,** 2520–2524.
6. Goldberg, A. R. & Hurwitz, J. (1972) *J. Biol. Chem.* **247,** 5637–5645.
7. Ratner, D. (1976) *Nature (London)* **259,** 151–153.
8. Richardson, J. P., Grimley, C. & Lowery, C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1725–1728.
9. Das, A., Court, D. & Adhya, S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1959–1963.
10. Korn, L. J. & Yanofsky, C. (1976) *J. Mol. Biol.* **106,** 231–241.
11. Inoko, H., Shigesada, K. & Imai, M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1162–1166.
12. Guarente, L. P., Mitchell, D. H. & Beckwith, J. (1977) *J. Mol. Biol.* **112,** 423–436.
13. Guarente, L. P. & Beckwith, J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 294–297.
14. Das, A., Adhya, S., Miller, W. & Merril, C. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 1401.
15. Shapiro, J. & Adhya, S. (1969) *Genetics* **62,** 249–264.
16. Burgess, R. R. (1969) *J. Biol. Chem.* **244,** 6168–6176.
17. Rosenberg, M., Weissman, S. & deCrombrugghe, B. (1975) *J. Biol. Chem.* **250,** 4755–4764.
18. Conway, T. W. & Lipmann, F. (1964) *Proc. Natl. Acad. Sci. USA* **52,** 1462–1466.
19. Boyd, D. H., Zillig, W. & Scaife, J. (1974) *Mol. Gen. Genet.* **130,** 315–320.
20. Das, A., Court, D., Adhya, S. (1978) in *Molecular Basis of Host Virus Interactions,* ed. Chakravarty, M. (Science Press), in press.
21. Lowery-Goldhammer, C. & Richardson, J. P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2003–2007.
22. Das, A., Court, D., Gottesman, M. & Adhya, S. (1977) in *DNA Insertion Elements, Plasmids and Episomes,* eds. Bukhari, A., Shapiro, J. & Adhya, S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 93–97.
23. Inoko, H. & Imai, M. (1976) *Mol. Gen. Genet.* **143,** 211–221.