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Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in transcription termination factor rho

(rho ATPase/polarity suppression/*gal* operon/λ *N* gene/recombination)

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ABSTRACT **Polarity suppressor mutants that are conditional lethal for growth have been isolated in *E. coli* K12. The mutations map between the *ilv* and *cya* loci of the *E. coli* chromosome. Rho factor isolated from one of these *ts* mutants does not show transcription termination activity at any temperature tested; however, it is found to be temperature sensitive for its poly(C)-dependent ATPase activity. Unlike the previously known polarity suppressor mutants (*suA* and *psu*), the *rho* mutation suppresses all types of polarity. Other interesting properties of these mutants include ultraviolet sensitivity, recombination deficiency, and decreased ability to lysogenize temperate phages λ and P1. Our results suggest that rho has an essential function in the growth and normal physiology of cells.**

The rho_{ts} mutant allows the growth of phage λ defective in the *N* gene. This result supports the model that *N* gene product prevents transcription termination by antagonizing rho activity.

Since operons (1) are envisaged as discrete transcriptional units, which can be turned on or off at the promoter end, it is likely that transcription would stop at the promoter distal end of these units. The *gal* operon of *Escherichia coli* has three structural genes—*K* (galactokinase), *T* (galactose-1-phosphate uridylyltransferase), and *E* (UDP-galactose-4-epimerase)—and an operator-promoter region located at the *E* end (2–4). The protein factor rho, which causes termination of transcription at specific termination sites on phage λ DNA template *in vitro* (5), also terminates transcription of *gal* at the end of the operon (6).

The phenomenon of polarity caused by mutations is manifested by a decrease in the levels of the products of genes located distal to the polar mutations (7, 8). Two distinct classes of mutations are known to have polar effects: nonsense mutations which cause premature translation termination, and DNA insertion mutations (9–11). Three types of DNA insertions have been well characterized: IS1, IS2, and IS3, containing about 700, 1400, and 1200 nucleotide pairs, respectively (12, 13).

We have proposed that all polarity is caused by premature termination of transcription by rho within an operon (ref. 14; S. Adhya, M. Gottesman, A. Das, B. de Crombrugghe, and D. Court, manuscript in preparation). Two observations support this idea: the existence of rho-sensitive termination signals within operons (6, 15) and existence of the signals within the polar DNA insertion IS2 (6). Several bacterial mutations (*suA* and *psu*) have been reported that suppress only the polarity of some mutations—nonsense mutations (16–20) and IS1 insertions—but not the polarity of IS2 and IS3 insertions (46). Supporting the transcriptional termination model of polarity (14), it has recently been shown that some of these polarity suppressor mutations affect rho (refs. 21–23; Korn and Yanofsky, personal communication). We report here the isolation and characterization of suppressor mutations of an insertion similar to IS3 in size, gal_3 (13). These suppressor mutations make the cell temperature sensitive for growth. One of these has been shown to result in a heat-labile rho protein and is able to suppress the polarity of various types of polar mutations.

Abbreviation: Gal^+ and Gal^-, ability and inability, respectively, to grow on galactose.

MATERIALS AND METHODS

Bacterial and Phage Strains. *E. coli* K12 strains employed in this work are described in Table 1. Wild-type and amber mutants of phage λ used were originally obtained from Allan Campbell (24). Phage P1*clr*100*Cam* was from Lee Rosner (25).

Media. Tryptone broth: 1% tryptone, 0.5% NaCl, 1 mM $MgSO_4$, and 1 μg/ml of thiamine hydrochloride. Tryptone agar: tryptone broth and 1.1% or 0.7% agar. L broth: tryptone broth, 0.5% Difco yeast extract, and 0.5% glucose. MacConkey agar described in the Difco Manual was used except that galactose or sorbitol was substituted for lactose (MacConkey sorbitol plates were used to score cya^+ transductants). All media were adjusted to pH 7.0 by NaOH. The minimal medium M56 (27) was used, supplemented with histidine and thiamine and 0.2% glucose, galactose, or glycerol. Minimal agar plates contained 2% agar.

Isolation of gal_3 Suppressors. Cells of SA1030 grown overnight in tryptone broth were resuspended in 10 mM $MgSO_4$ and approximately 10^8 cells were spread on a minimal galactose agar plate. A few crystals of *N*-methyl-*N'*-nitrosoguanidine (Aldrich Chemical Co., Wisc.) were placed at the center of the plate and the plate was incubated for 48 hr at 32°. Galactose-positive (Gal^+) revertants were observed around a clear zone of killing by the mutagen. After purification of the revertants on minimal galactose plates, 35 colonies were tested for heat sensitivity on tryptone agar plates.

Purification of Rho Factor. Rho factor was purified from strain AD1600 following the procedure described by Roberts (5). Cells were grown to mid log phase at 28° in L broth containing 0.5% glucose. A pure preparation of rho was obtained (see *Results*), even when the DEAE-cellulose step was omitted from the purification procedure. During storage at 4° of the glycerol gradient fractions, the poly(C)-dependent ATPase activity was as stable as that of the wild type. Purified wild-type rho and RNA polymerase were kindly provided by B. de Crombrugghe and R. Musso. Gel electrophoresis was kindly performed by B. Bhattacharyya.

Enzyme Assays. Transcription termination and ATPase activities of rho were assayed using [^{3}H]CTP (Schwarz/Mann) and [γ-^{32}P]ATP (New England Nuclear) as described by Lowery-Goldhammer and Richardson (28). Galactokinase was assayed as described by Wilson and Hogness (29), using D-[^{3}H]galactose (New England Nuclear).

RESULTS

Isolation of mutants

The gal_3 mutation, originally isolated by Lederberg (30), is a polar insertion of the IS3 size located in or near the operator-

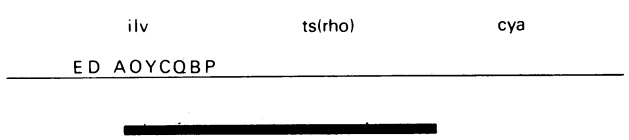

FIG. 1. Partial genetic map of rho and adjacent region of *E. coli* chromosome. *ilv* represents the genetic locus coding for enzymes of isoleucine and valine biosynthesis. *rho* represents the gene for transcription termination factor rho. *cya* is the gene for adenylate cyclase. *ts* represents the *ts*15 mutation described in this paper. The thick solid line shows the extent of the *E. coli* chromosome carried by the λ transducing phage.

promoter region of the *gal* operon (13) and does not mutate the structural genes (unpublished results). Based on the premise that insertional polarity is the result of rho-mediated transcription termination, we thought that a mutation in rho gene would make the gal_3 mutant phenotypically Gal$^+$. Spontaneous Gal$^+$ revertants of gal_3 arise by changes in the IS3 region (31, 32). We have isolated Gal$^+$ revertants of the gal_3 mutant, specifically induced by nitrosoguanidine at 32°. Sixteen out of 35 of these revertants tested were found to be heat sensitive for growth. We conclude that a single mutation is responsible for the suppression of gal_3 polarity and heat sensitivity of the cell, since heat-resistant revertants of these conditional lethal mutants are Gal$^-$.

Mapping of the *ts* mutations

Sixteen independent *ts* mutations map between the *ilv* and *cya* loci of *E. coli* chromosome (see Fig. 1). The F'14 episome, which carries the 74- to 79-min region of the chromosome (33), confers heat resistance and the Gal$^-$ character to the mutants. The *ts* alleles are, therefore, qualitatively recessive to the wild type. One *ts* mutation (*ts* 15) is cotransducible 67% (54/80) with an *ilv* marker and 87% (21/24) with a *cya* marker by phage P1 cotransduction. We have isolated a λ transducing phage, able to transduce the *ts*15 mutant to *ts*$^+$, from a lysate which was obtained by inducing a prophage located within the *ilvA* gene of the chromosome in strain KS506. This transducing phage, which has been found to transduce all the *ilv* markers located between the prophage and the *cya* gene, also transduces all 16 of the *ts* mutations. It, however, does not transduce a *cya* mutation. These results demonstrate the map order to be *ilv-ts-cya* (Fig. 1).

Table 1. List of bacterial strains used

Strain	Genotype	Reference or source
SA500	F$^-$ *his str*^{r} *sup*^{o}	(9)
SA1030	SA500 gal_3	(9)
S1654	SA500 *gal*Δ *sup*$^+$II	(9)
AD1600	SA1030 rho_{ts15}	This work
AD1601	SA1030 *ts*17	This work
AD1602	SA1030 *ts*18	This work
OR697	F$^-$*ilv* $trpE_{am}$ $lacZ_{ochreU118}$ (*gal-bio*)Δ	O. Reyes
SA1421	SA500 cya_{1026} (*gal-uvrB*)Δ	S. Adhya
DC47	F$^-$ *ilv his trp pro*/F'14 *ilv*$^+$ *supU*	L. Soll
KS506	HfrH (*gal-uvrB*)Δ with λcI_{857} located in *ilvA*	(26)
N100	W3102 *str*^{r} *sup*^{o} $recA_{13}$	M. Meselson

Rho factor from *ts*15 is heat labile for ATPase activity

We now present evidence that transcription termination factor rho is defective in a strain (AD1600) into which the *ts*15 mutation has been transferred by phage P1 transduction. It has recently been discovered that highly purified rho possesses a poly(C)-dependent ATPase activity (28). We have purified rho by the procedure of Roberts (5), but monitoring the rho protein by its ATPase activity. The ATPase activity of rho, isolated from both the wild-type and *ts*15 cells, was found to be absolutely dependent upon poly(C) at 32° (data not shown). We examined the ATPase reaction kinetics at different temperatures to determine if the rho protein isolated from the mutant shows heat-sensitive kinetics (Fig. 2). Whereas the ATPase activity of rho from wild-type cells increases with temperature up to 50°, that from the mutant strain showed a temperature optimum at 42°, with very little activity, if any, at 50°. The reaction rate of wild-type rho is linear at all temperatures, whereas rho from *ts*15 strain shows a decreasing rate at all temperatures. Note that the above effects of temperature on the ATPase activity of rho were studied when poly(C) and ATP were present during incubation at various temperatures. We have confirmed the observation of Lowery-Goldhammer and Richardson (28) that the ATPase activity of wild-type rho is inactivated if

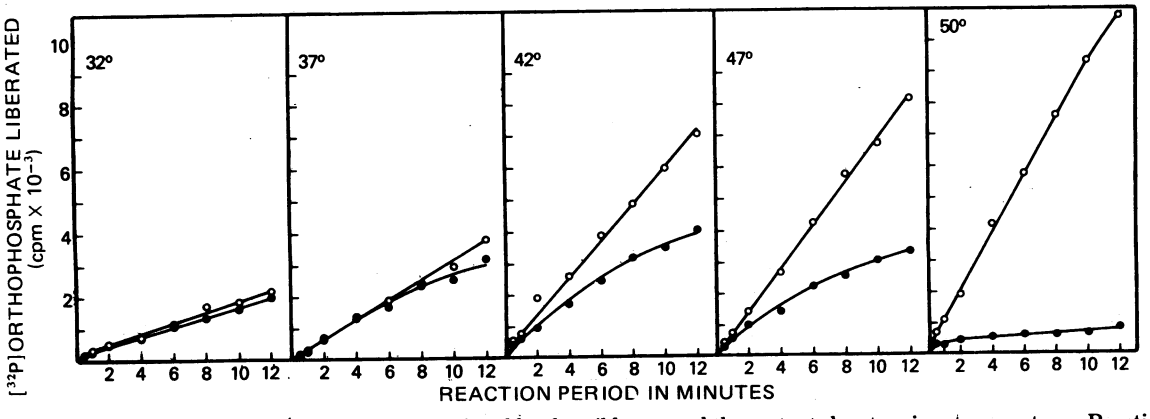

FIG. 2. Rate of poly(C)-dependent ATPase reaction catalyzed by the wild-type and the mutant rho at various temperatures. Reaction mixture (28) contained 40 mM Tris·HCl (pH 7.9), 50 mM KCl, 12 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetate (pH 8.0), 0.1 mM dithiothreitol, 0.7 mg/ml of bovine serum albumin, 2 μg/ml of poly(C), 0.4 mM ATP, and 0.28 μCi/μl of [γ-^{32}P]ATP. Reaction was started by adding 2 μg of rho (wild- type or mutant) in a 200 μl incubation mixture prewarmed at various temperatures. Samples (20 μl) were withdrawn at various times and put into 2 ml of chilled 20 mM sodium phosphate buffer (pH 6.0). Radioactivity in the orthophosphate liberated was determined from 1 ml of the solution after the removal of ATP by activated charcoal (34). Open circles and closed circles represent the wild-type and the mutant rho, respectively.

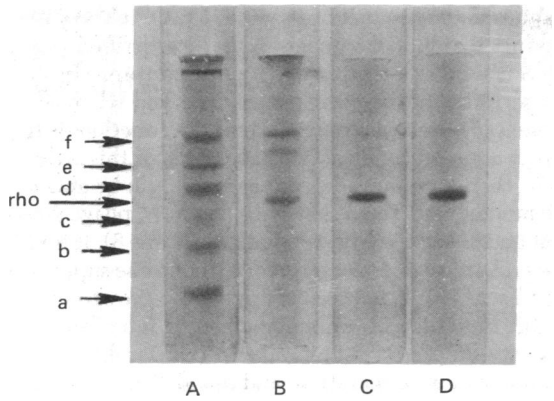

FIG. 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of mutant rho protein. Discontinuous polyacrylamide gels in presence of sodium dodecyl sulfate were run as described by Weber and Osborn (35). The gels contained 5% acrylamide and 0.2% bisacrylamide and were electrophoresed with 6 μg of mutant rho (36) at a current of 4 mA/tube at 23° until the tracking dye reached the end of the gel. Gels were stained with Coomassie Brilliant Blue. (A) Marker proteins (molecular weight): a, myoglobin (17,000); b, carbonic anhydrase (29,000); c, aldolase (40,000); d, glutamate dehydrogenase (55,000); e, transferrin (76,000); f, phosphorylase b (96,000). (B) Phosphocellulose fraction. (C) Glycerol gradient fraction. (D) Glycerol gradient fraction heated at 50° for 10 min.

preincubated at 47° in the absence of poly(C) and ATP. Moreover, we have found that rho from *ts*15 is inactivated much faster under these conditions (data not shown). We conclude that the *ts*15 mutation confers heat sensitivity to the ATPase activity of rho. An alternative possibility is that a minor protease contaminant is present in the mutant rho preparation. However, the presence of equal amounts of mutant rho (based on activity at 28°) and wild-type rho together during incubation at 50° gives a poly(C)-dependent ATPase activity equal to wild-type rho alone (data not shown). We also heated the mutant rho protein at 50° for 10 min and then electrophoresed in a polyacrylamide gel in the presence of sodium dodecyl sulfate (Fig. 3). There was neither any noticeable decrease in the intensity of the stain nor any increase in the mobility of rho protein.

Transcription termination and nascent-RNA-dependent ATPase activity of rho from *ts*15

Transcription termination activities of wild-type and mutant rho were compared. Termination activity was assayed by a decrease in net RNA synthesis in a reaction mixture containing DNA, RNA polymerase, and four ribonucleoside triphosphates with or without rho factors added (21). Two of the ribonucleotides were labeled: [^{3}H]CTP to measure the amount of RNA synthesis and [γ-^{32}P]ATP to follow the release of orthophosphate. The latter measures the nascent-RNA-dependent ATPase activity of rho (21), which has been shown to be essential for transcription termination activity (37). The results show that the mutant rho does not terminate transcription at any temperature tested, mimicking the results of a reaction without rho (see Fig. 4a). Increasing the mutant rho concentration up to 15-fold does not provoke termination (data not shown). Interestingly, the mutant protein, which has been shown to possess the poly(C)-dependent ATPase activity at lower temperatures, does not show measurable ATPase activity dependent on nascent RNA, like the transcription termination activity, at any temperature, under our conditions (Fig. 4b). The deficiency of transcription termination activity (Fig. 4a) of the mutant rho

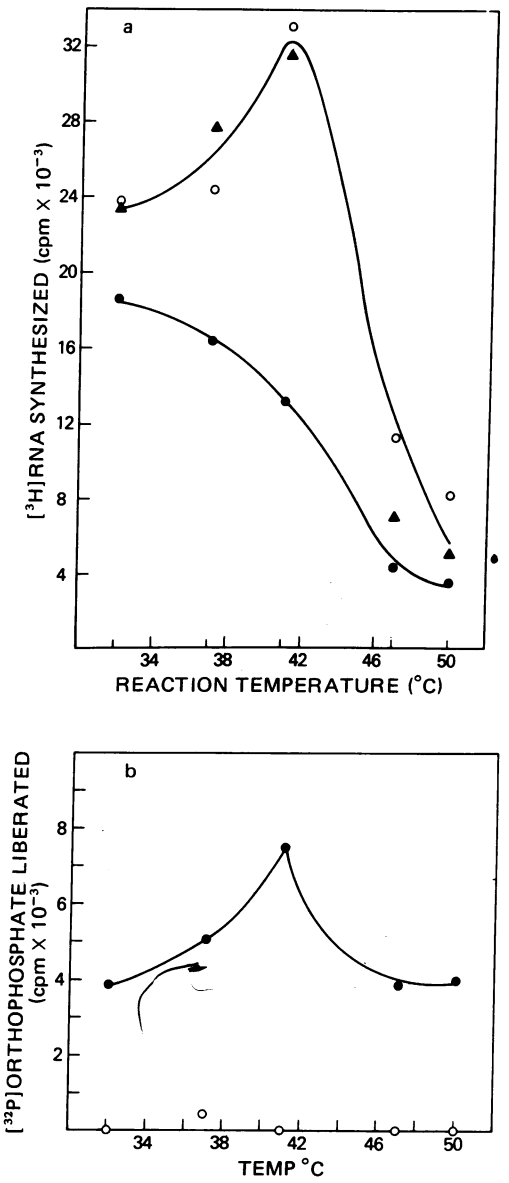

FIG. 4. Depression of RNA synthesis and the nascent-RNA-dependent ATPase activity of wild-type and mutant rho. Each reaction mixture contained 40 mM Tris·HCl (pH 8.0); 50 mM KCl; 12 mM $MgCl_2$; 0.1 mM ethylenediaminetetraacetate (pH 8.0); 0.1 mM dithiothreitol; 0.3 mg/ml of bovine serum albumin; 0.4 mM each of GTP, CTP, UTP, and ATP; 0.28 μCi/μl of [γ-^{32}P]ATP and 0.4 μCi/μl of [^{3}H]CTP; 0.5 μg of RNA polymerase; 2.4 μg of λ *gal*$_{313}$ DNA; and 0.4 μg of wild-type or mutant rho where indicated. In a total volume of 105 μl, the reaction was initiated by adding rho and RNA polymerase or RNA polymerase alone. After an incubation for 12 min at the indicated temperatures, reaction was terminated by chilling. Aliquots (50 μl) were used to measure orthophosphate (reaction without rho serves as the control at different temperatures) and the rest was used for measuring trichloroacetic-acid-insoluble radioactivity. Triangles represent the reaction without rho, solid circles with wild-type rho, and open circles with mutant rho.

even at 32° is consistent with the Gal$^+$ character of the rho mutant carrying the *gal*$_3$ mutation.

Pleiotropic properties of rho mutant

1. Temperature sensitivity: The *rho* $_{15}$ mutation is conditionally lethal for cell growth. The mutant forms colonies at an efficiency of $2\cdot10^{-3}$ at 37°, $6\cdot10^{-7}$ at 39° and $6\cdot10^{-8}$ at 42°, when compared to 32°. The generation time of the mutant is 150 min

Table 2. Levels of galactokinase in various *ts* mutants

Strain	Genotype	Galactokinase specific activity	
		Uninduced	Induced
SA500	gal^+	1.5	10.5
SA1030	gal_3	0.4	0.2
AD1600	gal_3 *ts*15	3.1	6.5
AD1601	gal_3 *ts*17	1.5	3.0
AD1602	gal_3 *ts*18	1.4	4.9

Strains AD1600, AD1601, and AD1602 were constructed from the original *ts* isolates by P1 transduction into SA1030. Galactokinase was induced by 5 mM D(+)-fucose for 60 min at 32° in minimal glycerol medium and assayed as described before (14), using D-[^{3}H]galactose. Specific activity of the enzyme is defined as nmol of D-[^{3}H]galactose 1-phosphate formed per min/ml of cells of $OD_{590\ nm}$ = 1.0.

compared to 105 min for the wild type in liquid glucose minimal medium at 32°. At 42°, the mutant grows for one generation before growth stops.

2. Ultraviolet sensitivity: All the *ts* mutants, including *ts*15, are sensitive to ultraviolet light, when compared to the wild type. Twenty temperature-resistant revertants of *ts*15, isolated at 42°, are Gal$^-$ and UV-resistant. Many revertants of *ts*15, isolated as UV-resistant, are Gal$^-$ and temperature-resistant as well.

3. Recombination deficiency: To study the effect of rho_{ts15} mutation on the recombination proficiency of the cell, the recombination frequency between two genetic markers in λ phages was measured under conditions where phage-mediated recombination was eliminated (38). Compared to the wild type, there was a 70-fold reduction in recombination frequency at 42° in the mutant. λ Mutants that do not grow in a *recA* host (39) have been found to grow in *ts*15 host, suggesting that the recombination defect of the mutant is not because of the deficiency of *recA* gene product.

4. Lysogeny of temperate phages: The *ts* mutations affect the ability of *E. coli* cells to form stable lysogens of temperate phages λ and P1 as measured at 32°. These phages form clear plaques on these hosts at 32°, compared to turbid plaques on the wild type as well as ts^+ revertants.

5. Suppression of gal_3: The *ts* mutants were isolated as Gal$^+$ derivatives of a strain with an insertion similar to IS3 in size (gal_3) in the *gal* operon. The amounts of galactokinase made in some of these *ts* mutants, including *ts*15, are shown in Table 2. Compared to the parent, all of them synthesized significant amounts of galactokinase at 32°, whether or not D(+)-fucose, the *gal* inducer, is present. Experiments to be presented elsewhere show that at least part of the galactokinase synthesis can be accounted for by transcription from a promoter other than the known *gal* promoter.

6. Growth of λ N^- phage: The growth of phage λ is dependent upon the product of its gene *N* (24). In the absence of *N* product many of the genes are not transcribed (40–42). If λ DNA is used as a template in a purified transcription system, transcription of the same genes is blocked when rho factor is present (5). Roberts proposed that the function of *N* product was to antagonize the action of rho (43). If this is the only role of *N*, then λ mutants defective in *N* might be able to grow in the mutant host. The results show that λ N^- phage grows in *ts*15 host and not in the wild-type parent (Table 3). It is known that the *ts*15 strain does not possess any nonsense suppressor mutation because it is unable to support the growth of λ carrying amber mutations in various other genes. It has been shown that polarity suppressor mutations *suA* (C. Dambly and D. Court, manuscript in preparation) and *psu* (19) and other host mutations *sun* (20) and *nit* (44) also allow the growth of λ N^- phage. Not all of these mutations, which map near the *ilv* locus, have been tested for rho activity (21–23).

DISCUSSION

We have demonstrated that the *ts*15 mutation alters the rho protein and, therefore, is located in the structural gene of the protein. Although no transcription termination activity of the mutant protein is detected at any temperature *in vitro* (Fig. 4a), the poly(C)-dependent ATPase activity of rho is heat labile (Fig. 2). Thus the *ts*15 mutation has directly affected the poly(C)-dependent ATPase activity. Since the ATPase activity is essential for transcription termination (37), we could not determine if the loss of termination activity is a direct or indirect effect of the mutation.

The rho_{ts} mutant is defective for growth at temperatures above 32°. The reason for this defect is not obvious. The absence of transcription termination activity in this strain could directly or indirectly affect cell survival. Termination of transcription may be essential for cellular processes like DNA replication or maturation of ribosomal and transfer RNAs. Alternatively, readthrough of transcription from one operon may cause interference in the expression of an adjacent essential operon that is transcribed in the opposite direction. If the cell death is because of the absence of transcription termination, we assume that the mutant cell has some termination activity at 32° *in vivo*. On the other hand, the cell death may not be related to the absence of transcription termination. The ATPase activity of the rho polypeptide, which is known to be essential for the transcription termination process (37), may also be involved in other cellular processes requiring ATP hydrolysis. Thus, the cell survival may be dependent upon the ATPase activity of rho, independent of transcription termination.

We find that rho_{ts} mutation confers pleiotropic properties in the mutant strain: ultraviolet sensitivity, recombination deficiency, and an inability to lysogenize phage λ. It is clear from reversion studies that a single mutation in rho is responsible for all these phenotypes. Interference of these cellular functions may be related to rho by mechanism(s) similar to that we dis-

Table 3. Growth of λ N^- phage in the rho_{ts} mutant

Host	Genotype	Plaque-forming efficiency of λ			
		N^+	N_{am7}	N_{am500}	$N_{am7,am53}$
S1654	sup^+ rho^+	1.0	1.0	1.0	1.0(s)
SA1030	sup^o rho^+	1.0	8×10^{-6}	10^{-5}	10^{-7}
AD1600	sup^o rho_{ts}	1.0(m)	1.0(t)	1.0(s)	5×10^{-4}(s)

All phage strains, except N_{am500}, carry the CI_{857} mutation. N_{am500} is in λ*vir* strain. Plates were incubated at 36°. (m), (t), and (s) denote medium, tiny, and small plaque size, respectively (tiny is the smallest). Undesignated plaques are of normal size.

cussed for the cell survival. A third component of the *recBC* DNase, in addition to the *recB* and *recC* gene products, has been identified (45). The component may be controlled by the rho gene.

A general model to account for polarity in gene expression has been proposed (ref. 14; S. Adhya, M. Gottesman, A. Das, B. de Crombrugghe, and D. Court, manuscript in preparation). Basically, the model states that all polarity is the result of rho-mediated transcription termination within an operon. rho_{ts15} mutation has been found to release the polar effect of every type of polar mutation tested: nonsense mutations, and IS1, IS2, and IS3 DNA insertions (in preparation). Several other nonsense polarity suppressor mutations have also been tested for their ability to suppress insertion polarity. All of these have also been found to suppress IS1, but none suppresses IS2 and IS3 polarity (46). Some of these polarity suppressors have altered rho (refs. 21–23; Korn and Yanofsky, personal communication). Since they do not affect the cell survival and do not confer many other pleiotropic properties observed in rho_{ts15} mutant, the difference in suppression spectrum must be attributed to the level of residual rho activity in these strains.

Only one (*ts*15) out of 16 heat-sensitive mutants reported here has been tested and shown to be a rho mutant. The others having similar phenotypes and carrying mutations in the same region of the chromosome, may also be defective in rho. In addition to rho, other proteins may influence polarity (S. Adhya, M. Gottesman, A. Das, B. de Crombrugghe, and D. Court, manuscript in preparation). One can attempt to identify these factors by isolating polarity suppressor mutants starting from strains diploid for the *rho* gene.

1. Jacob, F. & Monod, J. (1961) *J. Mol. Biol.* **3**, 318–356.
2. Buttin, G. (1963) *J. Mol. Biol.* **7**, 183–205.
3. Echols, H., Reznichek, J. & Adhya, S. (1963) *Proc. Natl. Acad. Sci. USA* **50**, 286–293.
4. Shapiro, J. A. & Adhya, S. L. (1969) *Genetics* **62**, 249–264.
5. Roberts, J. (1969) *Nature* **224**, 1168–1174.
6. de Crombrugghe, B., Adhya, S., Gottesman, M. & Pastan, I. (1973) *Nature New Biol.* **241**, 260–264.
7. Jacob, F. & Monod, J. (1961) *Cold Spring Harbor Symp. Quant. Biol.* **26**, 193–211.
8. Franklin, N. C. & Luria, S. F. (1961) *Virology* **15**, 299–311.
9. Adhya, S. & Shapiro, J. A. (1969) *Genetics* **62**, 231–247.
10. Shapiro, J. A. (1969) *J. Mol. Biol.* **40**, 93–105.
11. Jordan, E., Saedler, H. & Sterlinger, P. (1968) *Mol. Gen. Genet.* **102**, 353–363.
12. Fiandt, M., Szybalski, W. & Malamy, M. H. (1972) *Mol. Gen. Genet.* **113**, 223–231.
13. Ahmed, A. & Scraba, D. (1975) *Mol. Gen. Genet.* **136**, 233–242.
14. Adhya, S., Gottesman, M. & de Crombrugghe, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2534–2538.
15. Shimizu, N. & Hayashi, M. (1974) *J. Mol. Biol.* **84**, 315–335.
16. Beckwith, J. (1963) *Biochim. Biophys. Acta* **76**, 162–164.
17. Carter, T. & Newton, A. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2962–2966.
18. Morse, D. E. & Guertin, M. (1972) *J. Mol. Biol.* **63**, 605–608.
19. Korn, L. & Yanofsky, C. (1976) *J. Mol. Biol.*, in press.
20. Brunel, F. & Davison, J. (1975) *Mol. Gen. Genet.* **136**, 167–180.
21. Richardson, J. P., Grimley, C. & Lowery, C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1725–1728.
22. Ratner, D. (1976) in *RNA Polymerase*, eds. Chamberlin, M. & Losick, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), in press.
23. Ratner, D. (1976) *Nature* **259**, 151–153.
24. Campbell, A. (1961) *Virology* **14**, 22–32.
25. Rosner, J. (1972) *Virology* **49**, 679–689.
26. Shimada, K., Weisberg, R. A. & Gottesman, M. E. (1972) *J. Mol. Biol.* **63**, 483–503.
27. Gottesman, M. E. & Yarmolinsky, M. B. (1968) *J. Mol. Biol.* **31**, 487–505.
28. Lowery-Goldhammer, C. & Richardson, J. P. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2003–2007.
29. Wilson, D. & Hogness, D. (1966) in *Methods in Enzymology*, eds. Neufeld, E. & Ginsburg, V. (Academic Press, New York), Vol. 8, pp. 229–240.
30. Lederberg, E. M. (1960) in *Microbial Genetics, Tenth Symposium of the Society for General Microbiology*, eds. Hayes, W. & Clowes, R. C. (Cambridge University Press, New York), pp. 115–131.
31. Hill, C. & Echols, H. (1966) *J. Mol. Biol.* **19**, 38–51.
32. Ahmed, A. (1975) *Mol. Gen. Genet.* **136**, 243–253.
33. Ohtsubo, E., Lee, H., Deonier, R. & Davidson, N. (1974) *J. Mol. Biol.* **89**, 599–618.
34. Krakow, J. (1966) *J. Biol. Chem.* **241**, 1830–1839.
35. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
36. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
37. Howard, B. & de Crombrugghe, B. (1976) *J. Biol. Chem.* **251**, 2520–2524.
38. Shulman, M. J., Hallick, L. M., Echols, H. & Signer, E. R. (1970) *J. Mol. Biol.* **52**, 501–520.
39. Zissler, J., Signer, E. R. & Schaeffer, F. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 469–475.
40. Skalka, A., Butler B. & Echols, H. (1967) *Proc. Natl. Acad. Sci. USA* **58**, 576–583.
41. Kourilsky, P., Marcaud, L., Sheldrick, P., Luzzati, D. & Gros, F. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 1013–1020.
42. Kumar, S., Bovere, K., Guha, A., Hradecna, Z., Maher, V. M., Sr. & Szybalski, W. (1969) *Nature* **221**, 823–825.
43. Roberts, J. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 121–126.
44. Inoko, H. & Imai, M. (1976) *Mol. Gen. Genet.* **143**, 211–221.
45. Lieberman, R. P. & Oishi, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4816–4820.
46. Reyes, O., Gottesman, M. & Adhya, S. (1976) *J. Bacteriol.*, in press.