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Genetic Analysis of a Temperature-Sensitive *Salmonella typhimurium rho* Mutant with an Altered Rho-Associated Polycytidylate-Dependent Adenosine Triphosphatase Activity

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A conditional-lethal *rho* mutant of *Salmonella typhimurium* LT2 has been isolated. The mutation was selected as a suppressor of the polarity of an insertion sequence (IS)2-induced mutation (*gal*$_3$) carried on an F′ plasmid. In addition to suppression of IS2-induced polarity, the *rho-111* mutation suppressed nonsense and frameshift polarity. The rho-associated polycytidylic acid-dependent adenosine triphosphatase activity in the mutant strain was elevated 15-fold above that in the parental strain, and the mutant rho protein was thermally unstable. A temperature-resistant revertant of the mutant strain did not suppress polarity and contained normal levels of polycytidylic acid-dependent adenosine triphosphatase, suggesting that the phenotype of the *rho-111*-bearing strain is the consequence of a single mutation. The *rho-111* mutation was located on the *S. typhimurium* linkage map midway between the *ilv* and *cya* loci by phage P22 cotransduction studies. F′ plasmid maintenance was not impaired in the mutant strain, and the mutation was recessive to the wild-type allele. The *rho-111* mutation did not alter in vivo expression of either the tryptophan or histidine operons.

In operons, mutational polarity refers to the reduction of gene expression for cistrons operator-distal to the cistron with the polar mutation. Numerous suppressors of polarity that do not suppress the original polarity-inducing mutation have been isolated in *Escherichia coli* (reviewed by Adhya and Gottesman [1]). Many polarity suppressor mutants are altered in the transcription termination protein rho, initially described by Roberts (31), or in the rho-associated polycytidylate [poly(C)]-dependent ATPase (24), or in both. Conditional-lethal *rho* mutants have been described. One mutant, *rho-15*(Ts), was isolated as a suppressor of the rho-dependent termination signal in a DNA insertion sequence, IS2, located in the galactose operon leader sequence (*gal*$_3$ [9]). The *rho-15*(Ts) mutant has a pleiotropic phenotype. The mutant is sensitive to UV irradiation, is deficient in generalized recombination, and fails to grow on malate or succinate as sole carbon sources. It is defective in establishment of phage P1 and lambda lysogens (9) and in maintenance of F and resistance plasmids (4).

Studies with the histidine operon of *Salmonella typhimurium* have contributed to our understanding of polarity (6). Recently, the DNA sequences of the transcription terminators in the *S. typhimurium his* (3) and *trp* (23) attenuator regions have been determined. The *E. coli* and *S. typhimurium trp* attenuators differ in that in vitro transcription termination at the *S. typhimurium trp* attenuator is less efficient than that at the *E. coli trp* attenuator (23). Although some *E. coli rho* mutants appear to suppress *trp* attenuation in vivo (21), attenuation in vitro is rho independent (23). To date, no *rho* mutants have been isolated in *S. typhimurium*. Hence, the effect of an altered rho factor on in vivo attenuation in the *S. typhimurium his* and *trp* operons is not known.

In this communication, we describe the isolation and properties of a conditional-lethal *S. typhimurium rho* mutant. (Results presented in this paper were taken from a Ph.D. thesis presented by P.R.H. to The University of Michigan. Preliminary accounts of this work have been presented previously [P. R. Housley, A. D. Leavitt, and H. J. Whitfield, Genetics **91**:s51, 1979; P. R. Housley and H. J. Whitfield, Fed. Proc. **39**:2202, 1980].)

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this work are listed in Table 1. *S. typhimurium* strains are derivatives of strain LT2. *E. coli* strains are derivatives of strain K-12.

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TABLE 1. *Bacterial strains*

Strain	Genotype[a]	Origin, comment[b]
E. coli		
AA3258	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$^+$)/Δ(*nadA aroG gal*) *thi-1 rpsL104 recA1*	F'8/W3104 × PL225, *recA* by conjugation
AA3512	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$_3$ *zzf*^{c}::Tn*5*)/Δ(*nadA aroG gal*) *thi-1 rpsL104 recA1*	AA8501 × PL225, *recA* by conjugation
AA8501	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$_3$)/*gal*$_3$ *thr-1*::Tn*5*	*thr-1*::Tn*5*(P1) × W4611D by H. Miller
F'8/W3104	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$^+$)/*galT12*	CGSC 2605[d]
PL225 *recA*	F$^-$ Δ(*nadA aroG gal*) *thi-1 rpsL104 recA1*	CGSC 5227
W4611D	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$_3$)/*gal*$_3$	H. Echols (18)
S. typhimurium		
AA111	F$^-$ *metE338 hisGo1242 hisC2124 trpE49 amtA1 ara-9 rho-111*	H111 (P22) × AA464
AA115	F$^-$ *metE338 hisGo1242 hisC2124 trpE49 amtA1 ara-9 cya-4 rho-111*	Spontaneous fosfomycin-resistant derivative of AA111
AA116	F$^-$ *ilvE401 metE338 trpE49 amtA1 ara-9 cya-2 rho*$^+$[e]	Spontaneous fosfomycin-resistant derivative of AA424
AA410	F$^-$ *metE338 hisGo1242 hisC2124 trpE49 amtA1 ara-9*	LT2 (P22) × AA464; Ilv$^+$ transductant
AA411	F$^-$ *ilvE401 hisGo1242 hisC2124 trpE49 amtA1 ara-9*	LT2 (P22) × AA464; Met$^+$ transductant
AA421	F$^-$ *metE338 trpE49 amtA1 ara-9 rho-111*	LT2 (P22) × AA111; His$^+$ transductant
AA424	F$^-$ *ilvE401 metE338 trpE49 amtA1 ara-9 rho*$^+$	LT2 (P22) × AA464; His$^+$ transductant
AA454	F$^-$ *ilvE401 metE338 hisD2421 trpE49 amtA1 ara-9 rho*$^+$	This study; strain AA454 is derived from strain TA6 (15) via several steps
AA464	F$^-$ *ilvE401 metE338 hisGo1242 hisC2124 trpE49 amtA1 ara-9 rho*$^+$	*hisO1242 hisC2124* (P22) × AA454; Hol$^+$ transductant
AA557	F$^-$ Δ(*nadA aroG gal bio uvrB chl dhb*) *spc-1*	Spontaneous Spc^r derivative of TA1674 (2)
AA558	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$^+$)/Δ(*nadA aroG gal bio uvrB chl dhb*) *spc-1*	AA3258 × AA557 by conjugation
AA830	F$^-$ *metE338 cya-1 hisGo1242 hisC2124 amtA1 ara-9 srl-202*::Tn*10 recA1 rho-111*	LT2 (P22) × AA922; Trp$^+$ transductant
AA921	F$^-$ *metE338 hisGo1242 hisC2124 trpE49 amtA1 ara-9 srl-202*::Tn*10 recA1 rho-111*	TT521 (P22) × AA111; Tet^r transductant
AA922	F$^-$ *metE338 cya-1 hisGo1242 hisC2124 trpE49 amtA1 ara-9 srl-202*::Tn*10 recA1 rho-111*	Spontaneous fosfomycin-resistant derivative of AA921
AA925	F$^-$ *ilvE401 cya-2 metE338 trpE49 amtA1 ara-9 srl-202*::Tn*10 recA1 rho*$^+$	TT521 (P22) × AA116; Tet^r transductant
AA926	F$^-$ *ilvE401 metE338 hisGo1242 hisC2124 trpE49 amtA1 ara-9 srl-202*::Tn*10 recA1 rho*$^+$	TT521 (P22) × AA464; Tet^r transductant
AA944	F$^-$ *ilvA595*::Tn*10 metE338 hisGo1242 hisC2124 cysB529 trpR-2 rho*$^+$	This study via several intermediate strains
AA945	F$^-$ *IlvA595*::Tn*10 metE338 hisGo1242 hisC2124 cysB529 trpR-2 rho-111*	This study via several intermediate strains
AL9	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$_3$ *zzf*::Tn*5*)/Δ(*nadA aroG gal bio uvrB chl dhb*) *hisGo1242 hisC2124 spc-1 rho*$^+$	This study; see text
H111	F'8(*nadA*$^+$ *aroG*$^+$ *gal*$_3$ *zzf*::Tn*5*)/Δ(*nadA aroG gal bio uvrB chl dhb*) *hisGo1242 hisC2124 spc-1 rho-111*	This study; see text
hisGo1242 hisC2124	F$^-$ *hisGo1242 hisC2124*	B. Ames
LT2	*ara-9*	Wild-type; B. Ames
(pDB450)/DU3	F' pDB450 (*ilv*$^+$ *rho*$^+$ *cya*$^+$ *metE*$^+$)/*ilvD18 ilvA2280 leuA409 srl-202*::Tn*10 recA1*	R. O. Burns (5)
TA6	F$^-$ *ilvE401 metE338 hisD2421 ara-9*	J. Roth (15)
TT521	F$^-$ *srl-202*::Tn*10 recA1 rpsL104*	J. Roth

[a] Genetic symbols are those of Sanderson and Hartman (33). The *his* operator, formerly *hisO*, is written *hisGo*.
[b] (P1) or (P22) indicates source of transducing lysate.
[c] Nomenclature conventions for insertion mutations follow the suggestions of Chumley et al. (8).
[d] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.
[e] The wild-type, nonsuppressing allele is designated *rho*$^+$.

Media. Minimal medium E and Difco nutrient medium for growth of bacterial strains and bacteriophage were prepared and supplemented when appropriate as described by Roth (32).

Materials. The suppliers of reagents were as follows: poly(C), P-L Biochemicals, Inc.; [γ-^{32}P]ATP, ICN Chemical and Radioisotope Division; L-histidinol phosphate and L-histidinol, Vega-Fox Biochemicals; chorismic acid and indole, Sigma Chemical Co.; and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine), Aldrich Chemical Co. All other chemicals were reagent grade.

Enzyme assays. Assays for the histidine biosynthetic enzymes, histidinol phosphate phosphatase (*hisB*; EC 3.1.3.15; L-histidinol phosphohydrolase) and histidinol dehydrogenase (*hisD*; EC 1.1.1.23; L-histi-

dinol-NAD oxidoreductase), were according to the methods of Ely (13) and Martin et al. (26), respectively. The tryptophan biosynthetic enzymes anthranilate synthase (*trpE*; EC 4.1.3.27; chorismate pyruvate-lyase [amino-accepting]) and tryptophan synthase (*trpB* and *trpA*; EC 4.2.1.20; L-serine hydro-lyase [adding indole-glycerol-phosphate]) were assayed as described by Wuesthoff and Bauerle (37) and Herschman and Helinski (17), respectively. Galactokinase (EC 2.7.1.6; ATP:D-galactose-1-phosphotransferase) was assayed by the method of Wetekam et al. (35), using toluene-treated cells (D. Friedman, personal communication). Poly(C)-dependent rho ATPase activity was determined as described by Lowery-Goldhammer and Richardson (24) in crude extracts prepared according to the method of Imai and Shigesada (19). For thermal stability studies, samples of crude cell extracts were preincubated at 50°C in the buffer described by Imai and Shigesada (19) before assay for poly(C)-dependent ATPase at 30°C. Protein was measured by the method of Lowry et al. (25) with bovine serum albumin as the standard.

For all six enzymes assayed, values reported are averages of at least two and usually three independent experiments. For each experiment, assays were done in duplicate or triplicate using different enzyme concentrations.

Genetic techniques. Phage lysates of phage P22 HT105/1 *int-201* (8) were prepared and used in transductions as previously described (11). Transductions with phage P1CM *clr-100* (chloramphenicol resistance), using P1-sensitive, Gal$^-$ *S. typhimurium* recipients, were carried out according to the methods of Mojica-A (28).

Recombinants arising from conjugation or transduction experiments were purified twice selectively, once nonselectively, and finally once selectively prior to verification for the appropriate phenotype. Only phage-sensitive transductants were saved for further study.

Generation times were determined from viable cell counts. The generation time for strain AA111 (*rho-111*) at 37°C in appropriately supplemented glucose minimal media was determined turbidimetrically.

Identification of polarity suppressor mutants. Polarity suppressor mutants were sought among the nitrosoguanidine-induced Gal$^+$ revertants of a strain harboring an F'8(*gal$_3$*) episome as described in the Results section. To identify polarity suppressor mutants, a strong polar mutation from an unmutagenized strain, *hisC2124*, was introduced into each Gal$^+$ revertant and the parental strain by the following two-step transduction procedure.

A phage lysate grown on strain TT47 (*hisD8557*::Tn*10*), containing a Tn*10* insertion in the *hisD* gene, was used to transduce each Gal$^+$ revertant and the parental strain to Tet^r and His$^-$. Since histidinol dehydrogenase, encoded by the *hisD* gene, converts histidinol (Hol) to histidine, these transductants are also Hol$^-$. The resulting Hol$^-$ Tet^r transductants were then used as recipients for a phage lysate prepared on a donor strain that contains the *his* operator-constitutive mutation *hisGo1242* and a strong polar frameshift mutation, *hisC2124* (16). Hol$^+$ transductants were selected, and those that were Tet^s and His$^-$ were assayed for histidinol dehydrogenase. Phage-sensitive, Tet^s His$^-$ transductants which contained constitutive levels of histidinol dehydrogenase had acquired the *hisGo1242 hisC2124* operon, and these transductants were assayed for histidinol phosphate phosphatase (*hisB*).

RESULTS

Isolation and initial characterization of a polarity suppressor mutant in *S. typhimurium*. We have applied an adaptation of the polarity suppressor mutant selection devised by Das et al. (9) to *S. typhimurium*. An F'8(*nadA*$^+$ *aroG*$^+$ *gal$_3$*) episome carrying the *gal$_3$*, IS2 insertion mutation was introduced into an *S. typhimurium* strain containing an extensive deletion of the chromosomal region including the *gal* operon and the surrounding region [Δ(*nadA-aroG-gal-bio-uvrB-chl-dhb*), Fig. 1]. Polarity suppressor mutants were sought among the nitrosoguanidine-induced Gal$^+$ revertants at 30°C. Of 150 Gal$^+$ revertants isolated at 30°C, 12 were temperature sensitive for growth on rich media at 42°C. To identify polarity suppressor mutants, a histidine operon carrying a strongly polar frameshift mutation in the *hisC* gene (*hisC2124*) was introduced into each temperature-sensitive Gal$^+$ revertant and into the parental strain by transduction as described in Materials and Methods. Suppression of polarity was measured by determining the activity of histidinol phosphate phosphatase, encoded by the *hisB* gene located operator-distal to the *hisC* gene. The fact that the histidine operon genetic material originates from an unmutagenized strain ensures that any apparent suppression of *hisC2124* polarity is not merely a consequence of nitrosoguanidine-induced multiple mutations altering the histidine operon itself (7). Two of the 12 Gal$^+$ revertants contained threefold higher levels of *hisB* as compared to the parental strain and remained phenotypically His$^-$. One revertant, designated strain H111 and containing the *rho-111* mutation, was chosen for further study.

The level of suppression of the *gal$_3$* mutation was quantified by measuring galactokinase activity encoded by the *galK* gene (Table 2). *S. typhimurium* AA558 carrying the wild-type F'8(*gal*$^+$) episome expresses normal, inducible levels of galactokinase (Table 2). Strain AL9, which carries the F'8(*gal$_3$*) episome, has a Gal$^-$ phenotype and expresses very low, constitutive levels of galactokinase (Table 2). *E. coli* strains harboring the F'8(*gal*$^+$) or F'8(*gal$_3$*) episomes express levels of galactokinase in the range of that found in *S. typhimurium* strains AA558 or AL9, respectively (data not shown). In contrast, strain H111 (*rho-111*) has a Gal$^+$ phenotype and

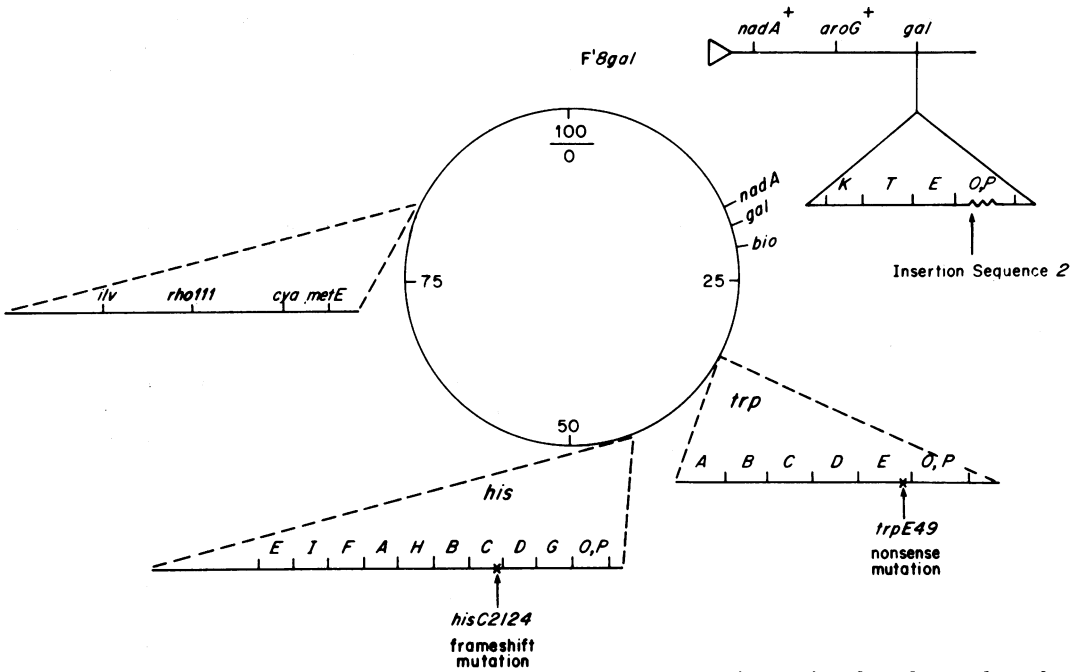

FIG. 1. *Chromosome of S. typhimurium with relevant markers. Appropriate regions have been enlarged to indicate relative positions of markers or cistrons within the his, trp, and gal operons. Markers on the F'8 episome are also indicated.*

TABLE 2. *Suppression of IS2-induced polarity in the gal operon by the rho-111 mutation*[a]

Strain	Pertinent genotype or source[b]	Galactokinase sp act[c]	
		Uninduced	Induced[d]
AA558	F'8(*gal*$^+$)/ Δ*gal rho*$^+$	7.30 ± 0.65	50.1 ± 4.30
AL9	F'8(*gal*$_3$)/Δ*gal rho*$^+$	0.70 ± 0.01	0.80 ± 0.10
H111	F'8(*gal*$_3$)/Δ*gal rho-111*	12.6 ± 0.50	22.1 ± 1.80
AA557	Δ*gal rho*$^+$	0.1	0.1

[a] The *gal*$_3$ mutation is a polar insertion of IS2 in the operator-promoter region of the *gal* operon which does not alter the structural genes (9). Strains containing the *gal*$_3$ mutation and the *rho*$^+$ allele are phenotypically Gal$^-$, whereas those with the *gal*$_3$ mutation and the *rho-111* allele are phenotypically Gal$^+$.

[b] Only the relevant genotype is shown. Complete genotypes are given in Table 1.

[c] Galactokinase specific activity, nanomoles of D-[1-^{14}C]galactose 1-phosphate formed per minute per milliliter of cells at OD_{650} = 1, average ± standard deviation. Galactokinase was assayed according to the method of Wetekam et al. (35), using toluene-treated cells (D. Friedman, personal communication). Assay results are given as initial rates. Cells were grown at 30°C in minimal medium E without citrate supplemented with 0.5% glycerol, 0.6% Casamino Acids, and appropriate auxotrophic requirements. Assays were conducted at 37°C.

[d] For induction, cells were grown in 5 mM D-(+)-fucose for five generations.

contains constitutive levels of galactokinase approximately 15-fold above that found in the parental strain, AL9 (*rho*$^+$). Constitutive expression of *gal* enzymes due to suppression of insertion mutations in the operator-promoter region has been observed in *E. coli* (9).

Two experiments demonstrate that strain H111 (*rho-111*) is Gal$^+$ as a result of a chromosomal mutation. The first experiment shows that the *gal*$_3$ mutation on the F'8(*gal*$_3$) episome in strain H111 (*rho-111*) is still present. The F'8(*gal*$_3$) episomes from the parental strain AL9 (*rho*$^+$) and mutant strain H111 (*rho-111*) were each transferred to a *recA* strain of *E. coli* deleted for the *nadA bio gal* region of the chromosome. Nic$^+$ Lac$^+$ transconjugants were selected and then scored for the unselected Gal phenotype. Of thirty Nic$^+$ Lac$^+$ transconjugants arising from the mating with the phenotypically Gal$^+$ donor strain H111 (*rho-111*) tested, all were Gal$^-$. As expected, transconjugants arising from matings with either strain AL9 (*rho*$^+$) or H111 (*rho-111*) as donor have the low polar galactokinase levels characteristic of the *gal*$_3$ mutation (data not shown).

The following experiment shows that the mutation responsible for the Gal$^+$ phenotype of strain H111 (*rho-111*) is located on the chromosome near the *ilv* operon. Since *rho* mutants in *E. coli* are located near the *ilv* region, a Tn*10* transposon inserted in the *ilv* operon was placed near the presumed location of the *rho-111* mutation. First a transducing lysate prepared on strain TT58 (*ilvA595*::Tn*10*) was used to trans-

duce strain H111 (*rho-111*) to Tet^r. A Tet^r Ilv$^-$ Gal$^+$ transductant was then used as a donor in a transduction with the parental strain AL9 (*rho*$^+$) containing the F'8(*gal*$_3$) episome as recipient. Of 115 Tet^r Ilv$^-$ transductants tested, 9 were Gal$^+$ and temperature sensitive for growth at 42°C. Therefore the mutation responsible for the Gal$^+$ phenotype and temperature-sensitive growth is linked to the *ilv* region of the chromosome.

To investigate further the properties of the *rho-111* mutation, we transferred the mutation into an unmutagenized strain, AA464. Strain AA464 contains polar mutations in the *trp* (*trpE49*) and *his* (*hisC2124*) operons. The *rho-111* mutation was introduced into strain AA464 by screening Ilv$^+$ transductants for temperature-sensitive growth. One temperature-sensitive, phage-sensitive transductant, designated strain AA111 (*rho-111*), was chosen for additional study.

Further characterization of the *rho-111* mutation. (i) Growth properties. The presence of the *rho-111* mutation results in a temperature-sensitive phenotype. Compared to the wild-type strain AA464 (*rho*$^+$) at 30°C, the mutant strain AA111 (*rho-111*) exhibits an efficiency of colony formation at 42°C of 6×10^{-5} on nutrient medium and less than 10^{-7} on appropriately supplemented minimal medium. When generation times were determined in several media, similar values were obtained for wild-type and mutant strains at 30°C in nutrient broth medium (53 and 49 min, respectively) and in appropriately supplemented minimal media with glucose (83 and 90 min, respectively) or glycerol (94 and 100 min, respectively) as carbon sources. However, at 37°C the generation times of the wild-type strain are 25, 50, and 62 min, whereas those of the mutant strain are 37, 61, and 97 min, in rich, glucose minimal, and glycerol minimal media, respectively. In glucose medium at 37°C, strain AA111 (*rho-111*) does not divide and instead produces long filaments. An *E. coli* temperature-sensitive rho mutant, *nitA702*, described by Imai and Shigesdada (19), is also defective in cell division.

(ii) Suppression of mutational polarity. Mutations in the first structural gene of the *trp* operon, *trpE*, result in auxotrophy for tryptophan which can be replaced by low concentrations of anthranilic acid. Polar *trpE* mutants are inhibited by high concentrations of anthranilic acid, presumably due to inhibition of one of the *trp* enzymes which already is expressed at a low, polar level (29). Strain AA111 contains the *rho-111* mutation and the strongly polar ochre mutation *trpE49* (37). The polarity of the *trpE49* mutation is relieved by the *rho-111* mutation as judged by resistance of strain AA111 to high concentrations of anthranilic acid. The resistance to anthranilic acid is not due to internal *trp* events since strain AA111 remains phenotypically Trp$^-$ and reverts to Trp$^+$.

In the *his* operon, polar *hisC* mutations reduce expression of the operator-distal *hisB* gene as indicated by low levels of *hisB* enzyme (histidinol phosphatase). Data presented in Table 3 demonstrate that the *rho-111* mutation partially relieves the polarity of an amber and a frameshift mutation in the *hisC* gene. Suppression of nonsense and frameshift polarity is indicated by increased levels of histidinol phosphate phosphatase (lines 3 and 5, Table 3) and by persistence of the His$^-$ phenotype.

(iii) Crude extract poly(C)-dependent ATPase assays of strain A111 (*rho-111*) and analysis of temperature-resistant revertants. For the cases examined, *E. coli* polarity suppressor mutants are defective in either rho-mediated transcription termination or its associated poly(C)-dependent ATPase activity (reviewed in reference 1), or in both. Some polarity suppressor mutants actually contain elevated levels of poly(C)-dependent ATPase (9, 19, 30), presumably a reflection of the autogenous regulation of rho biosynthesis. Poly(C)-dependent ATPase activity in the mutant strain AA111 is approximately 15 times higher than that found in the isogenic wild-type strain AA464 (*rho*$^+$ [lines 1 and 2, Table 4]). Moreover, the mutant strain poly(C)-dependent ATPase activity is temperature labile in comparison to that of the wild-type strain (Fig. 2). The purified wild-type and mutant rho proteins have been added to crude extracts prepared from the wild-type or mutant strains. Results of these mixing experiments demonstrate that crude extracts of the mutant strain do not contain an inactivating component which alters the activity of either mutant or wild-type rho protein at 50°C. Additionally these mixing experiments also demonstrate that wild-type crude extracts do not have increased levels of an inhibitor of poly(C)-dependent ATPase activity (data not shown). Purification of rho protein from the parental and mutant strains has confirmed the notion that strain AA111 has increased amounts of a defective rho protein. Details of the biochemical defects of the mutant rho protein will be published elsewhere (Housley and Whitfield, manuscript in preparation).

To determine whether the temperature-sensitive growth, polarity suppression, and increased poly(C)-dependent ATPase activity are the result of a single mutation, 15 temperature-resistant revertants were isolated and analyzed. Strain AA101 is representative of the majority

TABLE 3. *Suppression of frameshift and nonsense polarity in the histidine operon*[a]

Strain	Relevant genotype[b]	Enzyme activity		Polarity value[c]
		hisD (relative sp act)[d]	*hisB* (sp act)[e]	
AA413	*hisGo1242 rho*$^+$	1.00	21.9 ± 2.45	1.00
AA464	*hisGo1242 hisC2124 rho*$^+$	0.86 ± 0.04	1.84 ± 0.06	0.10
AA111	*hisGo1242 hisC2124 rho-111*	0.86 ± 0.07	7.01 ± 0.53	0.36
AA414	*hisGo1242 hisC2450 rho*$^+$	1.02 ± 0.09	6.09 ± 0.39	0.27
AA415	*hisGo1242 hisC2450 rho-111*	1.01 ± 0.06	11.4 ± 2.98	0.51

[a] Suppression of *hisC2124* (frameshift mutation [16]) and *hisC2450* (amber mutation [J. Roth, personal communication]) polarity was measured by determining the activity of the genes operator proximal (*hisD*) and operator-distal (*hisB*) to the *hisC* gene. Gene order in the histidine operon is *oGDCBHAFIE*. Strains containing the *hisC2124* or *hisC2450* mutations are phenotypically His$^-$ regardless of the presence or absence of the *rho-111* allele.

[b] Only the relevant genotype is given; complete genotypes are listed in Table 1. Strains AA413, AA414, and AA415 have the same genetic background as strains AA464 and AA111.

[c] The polarity value is defined as the ratio of (*hisB* activity in *C* mutant/*hisB* activity in wild type) to (*hisD* activity in *C* mutant/*hisD* activity in wild type) (26).

[d] *hisD*: Histidinol dehydrogenase relative specific activity is expressed as units per milligram relative to that of strain AA413 (*hisGo1242*), average ± standard deviation. Histidinol dehydrogenase was assayed by the method of Martin et al. (26).

[e] *hisB*: Histidinol phosphate phosphatase specific activity, expressed as units of absorbancy at 820 nm per ml per 15 min per OD_{650} of cells, average ± standard deviation, was assayed as described by Ely (13).

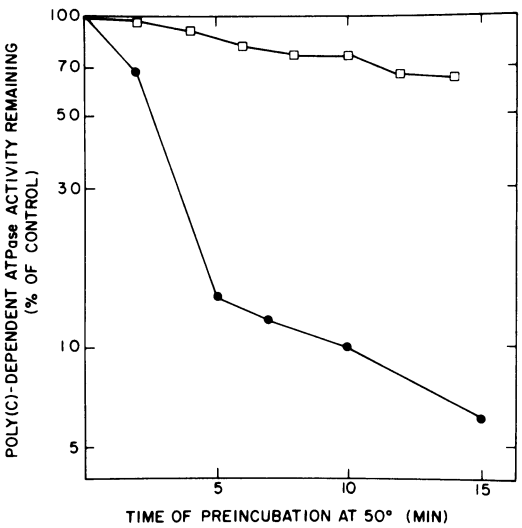

FIG. 2. *Heat stability of poly(C)-dependent ATPase activity. Assays were conducted as described in the text. Symbols: □, AA464 (rho*$^+$*); ●, AA111 (rho-111).*

of the revertants (Table 4). Although resistance to anthranilic acid is not altered qualitatively, the levels of *hisB* and poly(C)-dependent ATPase activity are intermediate between those of the mutant strain AA111 (*rho-111*) and wild-type strain AA464 *rho*$^+$). However, one temperature-resistant revertant, strain AA102 (Table 4), is now sensitive to anthranilic acid and has activities of *hisB* and poly(C)-dependent ATPase close to those found in the wild-type strain AA464. These results are consistent with the conclusion that the properties of the *rho-111* mutation result from a single mutational event.

(iv) Mapping of the rho-111 mutation. Phage P1 cotransduction data have localized many *E. coli* polarity suppressor mutations to the *ilv* region of the chromosome (reviewed in reference 1). The precise location of the *rho* gene has not been fully resolved. The *rho* mutations described by Das et al. (9) have been mapped between the *ilv* and *cya* genes, whereas those studied by Inoko et al. (20) appear to be located between the *cya* and *metE* genes. Yet, rho protein from both sets of mutants is defective in vitro transcription termination and in the rho-associated poly(C)-dependent ATPase activity. Thus precise mapping of the *rho-111* mutation using phage P22-mediated transduction might be useful in resolving this ambiguity. The results are summarized in Table 5 and Fig. 3. The *rho-111* mutation is 3% linked to *ilvE* and 4% linked to *cya* (Table 5). The *ilv* and *cya* genes are less than 0.1% cotransduced by phage P22 (Table 5). These results place the *rho-111* mutation between the *ilv* operon and *cya* gene, probably equidistant between these two loci. Note that by contrast the *hisR1223* mutation is 43% linked to the *cya* gene (line 7, Table 5). Hence the location of the *rho* gene is distinct from that of the *hisR* gene. Additional transduction studies using phage P1 confirm these results (Table 5 and Fig. 3) and are in agreement with P1 cotransduction studies in *E. coli* (9). The *rho-111* allele is 95% linked to the *cya* gene, whereas the *ilvE* and *metE* genes are only 0.5% linked (Table 5).

TABLE 4. *Characterization of temperature-resistant revertants of strain AA111 (rho-111)*[a]

Strain	Pertinent genotype or origin[b]	Growth phenotype		Enzyme sp act	
		Temperature sensitivity for growth at 42°C[c]	Sensitivity to anthranilic acid (200 μg/ml)[d]	*hisB*[e]	Poly(C)-dependent ATPase[f]
AA464	*rho*$^+$	R	S	1.84 ± 0.60	15 ± 3
AA111	*rho-111*	S	R	7.01 ± 0.53	247 ± 35
AA101	AA111[g]	R	R	4.37 ± 0.51	76 ± 11
AA102	AA111[g]	R	S	2.90 ± 0.27	23 ± 8

[a] Approximately 10^9 cells were incubated on appropriately supplemented minimal media plates for 4 days at 42°C. Colonies which appeared were purified by successive streaks at 42°C on supplemented minimal, nutrient, and supplemented minimal media before further characterization.

[b] Only the relevant genotype is shown. All strains are also *trpE49 hisGo1242 hisC2124*. Complete genotypes are given in Table 1.

[c] S, Temperature sensitive (mutant phenotype, *rho-111*) for growth at 42°C; R, temperature resistant (wild-type phenotype, *rho*$^+$) for growth at 42°C. Determined by streaking cells on nutrient agar plates and scoring growth at 30°C after 36 to 48 h of incubation and at 42°C after 16 to 20 h of incubation. Isogenic *rho*$^+$ and *rho-111* strains were included as controls.

[d] S, Sensitivity to 200 μg of anthranilic acid per ml (wild-type phenotype, *rho*$^+$); R, resistance to 200 μg of anthranilic acid per ml (mutant phenotype, *rho-111*). Anthranilic acid sensitivity was determined by visual inspection of colony size after 36 to 48 h of incubation at 30°C on appropriately supplemented minimal agar plates containing 200 μg of anthranilic acid per ml. The *rho-111* mutant cells (R) form 1- to 2-mm-diameter colonies, whereas *rho*$^+$ cells (S) form colonies that are 0.5 mm or less in diameter. Isogenic *rho*$^+$ and *rho-111* strains served as controls. Strains tested for response to anthranilic acid carry the strongly polar *trpE49* mutation, which severely reduces the expression of the distal *trp* enzymes. Anthranilic acid sensitivity is due to further inhibition of the activity of one of the distal *trp* enzymes (29). Suppression of the polarity of the *trpE49* mutation results in elevated expression of distal *trp* enzymes and resistance to high concentrations of anthranilic acid. Regardless of the presence (*rho-111*) or absence (*rho*$^+$) of the polarity suppressor allele, strains with the *trpE49* mutation retain their auxotrophy for either anthranilic acid (low concentrations) or tryptophan.

[e] *hisB*: Histidinol phosphate phosphatase specific activity, expressed as units of absorbancy at 820 nm per ml per 15 min per OD_{650} of cells, average ± standard deviation, was assayed by the method of Ely (13). Relief of the polarity caused by the *hisC2124* frameshift mutation was measured by assaying the level of histidinol phosphate phosphatase, encoded by the *hisB* gene which is downstream of the *hisC* gene. All strains in this table contain the *hisC2124* allele and are phenotypically His$^-$ regardless of the presence or absence of the *rho-111* polarity suppressor. Cells were grown at 30°C, and the *hisB* enzyme was assayed at 37°C.

[f] The RNA-dependent ATPase activity associated with the transcription termination protein rho was measured as the poly(C)-dependent release of orthophosphate from [γ-^{32}P]ATP in crude extracts as described by Imai and Shigesada (19). Specific activity is nanomoles per minute per milligram of protein, average value ± standard deviation. Cells were grown and assayed at 30°C.

[g] Temperature-resistant revertant of strain AA111.

(v) Dominance studies. To determine the dominance relationships of the wild-type and *rho-111* alleles, we constructed the series of monoploid and merodiploid strains outlined in Table 6. The F′(*ilv*$^+$ *rho*$^+$ *cya*$^+$ *metE*$^+$) factor carries *S. typhimurium* genetic material (R. O. Burns, personal communication; 5). All host strains are *recA1* to prevent recombination between chromosomal and plasmid-borne alleles and either Ilv$^-$ Met$^-$, Cya$^-$ Met$^-$, or Ilv$^-$ Cya$^-$ Met$^-$ to prevent segregation of the episome. Strain AA922 (*rho-111*), haploid for the *rho-111* allele, is temperature sensitive for growth, is resistant to high concentrations of anthranilic acid, and contains elevated levels of histidinol phosphate phosphatase (*hisB*) and poly(C)-dependent ATPase (Table 6). Strain AA822, the *rho*$^+$/*rho-111* merodiploid strain, exhibits the wild-type phenotype of the *rho* allele, namely, absence of temperature-sensitive growth, sensitivity to high concentrations of anthranilic acid, and low polar levels of *hisB* (Table 6). However, the poly(C)-dependent ATPase activity is intermediate between that found in the haploid wild type (line 1, Table 6) and polarity suppressor (line 2, Table 6) containing strains. Loss of the episomal wild-type *rho* allele in a spontaneous Cya$^-$ Met$^-$ segregant of the merodiploid strain results in reappearance of the characteristics associated with the *rho-111* allele (line 5, Table 6).

Therefore, the temperature-sensitive growth and polarity suppression of the *rho-111* mutation are recessive to the wild-type allele. Persistence of intermediate levels of poly(C)-dependent ATPase in the *rho*$^+$/*rho-111* merodiploid strain suggests some continued defect in autogeneous regulation of rho biosynthesis. Possibly large amounts of defective rho protein might interfere

TABLE 5. *P22 and P1-mediated cotransduction frequencies of genes in the rho region*[a]

Donor	Recipient	Selected marker	Unselected marker	% Linkage (no. of transductants/total)
P22 transduction				
AA111 (*rho-111*)	AA464 (*ilvE401*)	Ilv$^+$	Rho$^-$[b]	3 (7/239)
AA464 (*rho*$^+$)	AA115 (*cya-4*)	Cya$^+$[c]	Rho$^+$	4 (4/94)
SA1475 (*ilvA99*)	AA115 (*cya-4*)	Cya$^+$	Ilv$^-$	<0.1 (0/723)
LT2	AA111 (*metE338*)	Met$^+$	Rho$^+$	<0.4 (0/270)
AA115 (*cya-4*)	DU201 (*ilvC8*)	Ilv$^+$	Cya$^-$	<0.2 (0/600)
LT2	AA115 (*cya-4*)	Cya$^+$	Met$^+$	11 (6/56)
TA790 (*hisR1223*)	AA116 (*cya-2*)	Cya$^+$	HisR$^-$	43 (24/56)
P1 transduction				
AA183	AA185 (*ilvE401*)	Ilv$^+$	Met$^-$	0.5 (2/395)
AA183	AA180 (*cya-4*)	Cya$^+$	Rho$^+$	96 (96/100)

[a] P22-mediated transduction was performed using phage P22 HT105/1 *int-201* (8) as described by Davis et al. (11). P1-mediated transduction using phage P1CM *clr-100* was carried out with P1-sensitive strains as described by Mojica-A (28). Strains AA180, AA183, and AA185 are P1-sensitive derivatives of strains AA115, AA410, and AA411, respectively. Strains TA790 (*hisR1223*) and SA1475 (*ilvA99*) were obtained from B. N. Ames and K. E. Sanderson, respectively. Other genotypes are given in Table 1.

[b] Transductants were subjected to single-colony isolation and tested for polarity suppressor phenotype as reflected by growth at 30°C in the presence of 200 μg of anthranilic acid per ml (Ant^s or Ant^r) and by growth at 42°C on nutrient media. Strains that are wild type for the *rho* allele are Ant^s and grow at 42°C, whereas those containing the *rho-111* allele are Ant^r and do not grow at 42°C. Additional details are described in footnotes *c* and *d* of Table 4.

[c] Recombinants carrying Cya$^+$ were selected or scored for growth on media containing 0.4% ribose as the sole carbon source.

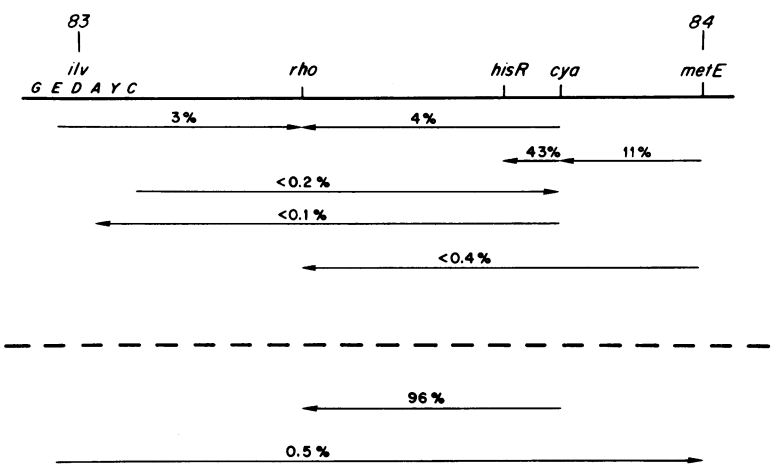

FIG. 3. *Genetic map of the 83 to 84 min region of the S. typhimurium chromosome. The numbers above the dotted line are P22 cotransduction frequencies (in percentages), and those below the dotted line are P1 cotransduction frequencies (in percentages), as reported in Table 5. The arrows point to the unselected markers.*

with the access of normal rho molecules to the site(s) for autogeneous regulation. Alternatively, mixing of wild-type and mutant rho protein subunits might yield hybrid rho oligomers partially defective in autogenous regulation.

Strain AA826, containing two copies of the wild-type *rho* allele, does not exhibit the expected twofold increase in poly(C)-dependent ATPase activity (Table 6). Transfer of the plasmid-borne *rho*$^+$ allele to another *rho*$^+$ recipient, strain AA925, confirmed this initial result. The poly(C)-dependent ATPase specific activity is again the same regardless of whether one or two copies of the *rho*$^+$ allele are present (lines 6 and 7, Table 6). Subsequent transfer of the episomal *rho*$^+$ allele from strain AA825 to another *rho-*

TABLE 6. *Characterization of strains monoploid or merodiploid for the rho allele*

Strain	Relevant genotype[a]	Ploidy of *rho* allele[b]	Growth phenotype		Enzyme sp act	
			Temperature sensitivity for growth at 42°C[c]	Sensitivity to anthranilic acid[d]	*hisB*[e]	Poly(C)-dependent ATPase[f]
AA926	*ilvE401 metE338 rho*$^+$	+	R	S	1.70 ± 0.23	8 ± 2
AA922	*cya-1 metE338 rho-111*	−	S	R	6.89 ± 0.44	147 ± 7
AA826	F′ (*ilv rho*$^+$ *cya*$^+$ *met*$^+$)/ *ilvE401 metE338 rho*$^+$	+/+	R	S	1.88 ± 0.36	9 ± 3
AA822	F′ (*ilv*$^+$ *rho*$^+$ *cya*$^+$ *met*$^+$)/*cya-1 metE338 rho-111*	+/−	R	S	1.96 ± 0.17	60 ± 6
AA823	*cya-1 metE338 rho-111*[g]	−	S	R	6.29 ± 0.44	153 ± 10
AA925	*ilvE401 cya-2 metE338 rho*$^+$	+	R	S	—[h]	7 ± 1
AA825	F′ (*ilv*$^+$ *rho*$^+$ *cya*$^+$ *met*$^+$)/ *ilvE4101 cya-2 metE338 rho*$^+$	+/+	R	S	—	9 ± 2
AA831	F′ (*ilv*$^+$ *rho*$^+$ *cya*$^+$ *met*$^+$/*cya-1 metE338 rho-111*	+/−	R	—	—	67 ± 12

[a] Only the pertinent genotype is shown. All strains are *recA1*. Strains AA822, AA825, and AA826 are derived from matings between the donor strain (pDB450)/DU3 and the recipient strains AA922, AA925 and AA926, respectively. Strain AA831 is derived from a mating between strain AA825 and strain AA830. Complete genotypes of the donor strain (pDB450)/DU3 and the recipient strains AA830, AA922, AA925, and AA926 are given in Table 1.

[b] Episomal *rho* allele/chromosomal *rho* allele. Nomenclature of *rho* alleles; +, *rho*$^+$; −, *rho-111*.

[c] S, Temperature sensitive (mutant phenotype, *rho-111* for growth at 42°C); R, temperature resistant (wild-type phenotype, *rho*$^+$ for growth at 42°C). Temperature sensitivity was determined as described in footnote *c* of Table 4.

[d] S, Sensitivity to 200 μg of anthranilic acid per ml (wild-type pheontype, *rho*$^+$); R, resistance to 200 μg of anthranilic acid per ml (mutant phenotype, *rho-111*). Anthranilic acid sensitivity was determined as described in footnote *d* of Table 4.

[e] *hisB*: Histidinol phosphate phosphatase specific activity, expressed as units of absorbancy at 820 nm per ml per 15 min OD_{650} of cells, average ± standard deviation. See footnote *e*, Table 4.

[f] Specific activity is expressed as the poly(C)-dependent release of orthophosphate (nanomoles per minute per milligram of protein at 30°C) from [γ-^{32}P]ATP, average ± standard deviation. See footnote *f*, Table 4.

[g] Spontaneous Cya$^-$ Met$^-$ segregant of strain AA822.

[h] —, Not tested.

111 recipient, strain AA830, resulted in strain AA831, which expressed intermediate levels of poly(C)-dependent ATPase (Table 6). Therefore, the episomal *rho*$^+$ allele is still functional.

(vi) Effect of the *rho-111* mutation on wild-type operon expression. Some *rho* mutants increase in vivo expression of the *trp* operon (21) and, under certain conditions, that of the *his* operon of *E. coli* (22). To evaluate any effects of the *rho-111* mutation on expression of the *trp* operon in *S. typhimurium*, we constructed an isogenic pair of strains, strains AA944 (*rho*$^+$) and AA945 (*rho-111*), containing a wild-type *trp* operon and a *trpR* mutation. Specific activities of anthranilate synthase, specified by *trpE* (29.9 ± 5.4 and 28.0 ± 0.2 nmol/min per mg), and tryptophan synthase, specified by *trpB* and *trpA* (5.98 ± 0.60 and 6.06 ± 0.42 nmol/min per ml per unit of optical density at 650 nm [OD_{650}] of cells), are identical in *rho*$^+$ and *rho-111* strains grown at 30°C in the presence of tryptophan. Similarly, the *rho-111* mutation has no major effect on expression of the wild-type *his* operon as judged by assays of histidinol dehydrogenase (*hisD*; 5.96 ± 0.80 and 4.97 ± 0.78 units/mg) and histidinol phosphate phosphatase (*hisB*; 0.88 ± 0.27 and 1.14 ± 0.19 units of absorbancy at 820 nm per ml per 15 min per OD_{650} of cells) on strains AA424 (*rho*$^+$) and AA421 (*rho-111*) grown at 30°C in histidine-supplemented media.

(vii) Plasmid maintenance. Our selection for the *rho-111* mutation demanded both suppression of IS2 polarity and maintenance of an F′ plasmid. To evaluate plasmid maintenance in *rho-111* strains, we prepared isogenic *rho*$^+$ and *rho-111* strains containing the F′42(*lac*$^+$) plasmid. Maintenance of the plasmid-borne Lac$^+$ phenotype was followed in nonselective media. The fraction of Lac$^+$ cells remained at 0.99 for both the *rho*$^+$ and *rho-111* strains after five generations of growth. Therefore, as might be expected, strains containing the *rho-111* mutation are not impaired in plasmid maintenance.

Finally, strains AA464 *rho*$^+$ and AA111 (*rho-111*) exhibit similar sensitivity to UV irradiation and grow equally well on malate or succinate as sole carbon sources, unlike the *E. coli rho-15*(Ts) mutant (9).

DISCUSSION

We have described the isolation and properties of a polarity suppressor mutant in *S. typhimurium*. The *rho-111* mutation confers a conditional-lethal phenotype on the cell, efficiently suppresses IS*2*-induced polarity, and suppresses nonsense and frameshift polarity. The rho-associated poly(C)-dependent ATPase activity in the mutant strain is thermally unstable in vitro and is elevated approximately 15-fold above that in the parental strain. The phenotype of the *rho-111* mutation is the consequence of a single mutation since a temperature-resistant revertant contains normal levels of poly(C)-dependent ATPase and no longer suppresses polarity. Thus we believe it is likely that the *rho-111* mutation is in the structural gene for rho. Phage P22 cotransduction frequencies place the *rho-111* mutation between the *ilv* and *cya* genes, probably equidistant from each locus. The conditional-lethal phenotype of the *rho-111* mutation is consistent with previous suggestions (9, 19) that rho is essential for cell viability.

The polarity suppressor selection employed in the present work requires continued maintenance of an F′ plasmid [F′8(*gal*$_3$)] in addition to suppression of the rho-dependent termination signal in IS*2* (14). Evidently the additional constraint of this selection resulted in an altered rho protein that is severly deficient in the autogenous regulation function as reflected by the significant elevation of poly(C)-dependent ATPase activity. Purification of rho protein from the parental and *rho-111* strains has confirmed that the level of rho protein in the mutant strain is 10- to 20-fold above that in the parental strain (Housley and Whitfield, manuscript in preparation).

In spite of the significant elevation of rho protein in the *rho-111* mutant, the effective in vivo transcription termination activity of the mutant strain must be sufficiently low to allow significant read-through at the rho-dependent terminator in the IS*2* insertion element (1, 14). Yet mutational polarity in the *hisC* gene is not as efficiently suppressed. Perhaps the natural *hisC* terminator uncovered as a result of the polar *hisC* mutation is somewhat rho independent. This postulated rho independence of the natural terminator sequence in the *hisC* gene might explain the failure of our earlier attempts at isolation of polarity suppressor mutants by using polar *hisC* mutants. Alternatively, the increased amounts of altered rho protein might cause at least some termination at this natural terminator sequence.

There is conflicting evidence concerning the role of rho in transcription termination at the attenuator region of the *his* and *trp* operons. Some *E. coli rho* mutants appear to affect expression of the *trp* operon in vivo by decreasing transcription termination at the attenuator. For example, the *psu-1*, *psu-2* (21), and *rho-15*(Ts) (cited by Adhya and Gottesman [1]) *rho* mutants increase *trp* operon expression two- to fourfold in *trpR* cells. The precise significance of these results is not clear since *trp* attenuation in vitro is rho independent (23). Additionally, the *psu-2* strain also carries a mutation, *trpX*, which mediates modification of tRNA^{Trp} (36). Furthermore, none of 10 different *rho* mutations, including the *psu-1*, *psu-2*, *rho-15*(Ts), and *rho-221* (isolated by Malamy, cited in reference 36) *rho* mutations, altered attenuation in the *E. coli* histidine operon (36). However in another strain background, the *rho-221* mutation did appear to increase expression of the *his* operator-proximal enzymes as much as fourfold when the cells were grown on glycerol as the sole carbon source (22).

The *rho* mutant described here does not significantly alter in vivo expression of the *S. typhimurium his* or *trp* operons. We have chosen to confine our enzyme assays to 30°C-grown cultures so as to minimize the influence of any secondary effects of the *rho-111* mutation at higher temperatures (36). Although the nucleotide sequences of the *E. coli* (12) and *S. typhimurium* (3) *his* attenuators are similar, the DNA sequence and properties of the *trp* attenuators in the two organisms are different. The calculated stability of the stem and loop structure immediately prior to the presumed point of transcription termination is lower for the *S. typhimurium* operon. The calculated ΔG value of this stem and loop structure is approximately −5 kcal (ca.−20.9 kJ) per mol for the *S. typhimurium* operon, whereas that for the *E. coli* operon is approximately −20 kcal (ca. −83.7 kJ) per mol (23). Additionally, in vitro transcription termination without added rho factor at the *S. typhimurium* attenuator is only 70% efficient as compared to termination at the *E. coli trp* attenuator, which is 95% efficient (23). Thus, in vivo attenuation in the *S. typhimurium trp* operon might be more sensitive to an altered rho factor. Therefore, it is noteworthy that expression of the *S. typhimirium trp* operon is not affected by the *rho-111* mutation.

The fact that strains carrying two copies of the wild-type *rho* allele do not contain the expected twofold increase in poly(C)-dependent

ATPase activity merits further comment. Absence of a gene dosage effect in strains diploid for a wild-type allele has been observed previously. Strains diploid for the structural gene (*ompA*) of a major outer cell envelope membrane protein of *E. coli* do not express a twofold increase in the level of the *ompA* protein (10). Expression of the *ompA* gene may be autogenously regulated (34). In the present work, there was no gene dosage effect in strains diploid for the wild-type *rho* allele, and the mutant strain contained significantly elevated levels of poly(C)-dependent ATPase activity. Both of these results are features that might be expected in the autogenous regulation of an abundant protein (M. A. Savageau, personal communication). This statement is based on two assumptions: (i) that rho protein is abundant relative to its binding constant for the site(s) involved in regulation of rho biosynthesis, and (ii) that a multimeric form of rho (greater than two subunits) participates in autogenous regulation. There are some experimental data supporting the first assumption. rho is an abundant protein, accounting for approximately 0.1% of the total cellular protein (19; Housley and Whitfield, manuscript in preparation). The approximately 15-fold increase in rho protein in the mutant strain also suggests that the wild-type protein is abundant relative to its binding constant and that the *rho* gene is maximally repressed under normal circumstances. Confirmation of these ideas will require further experimental data.

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