RHO AND RIBOSOME MUTATION INTERACTION: LETHALITY OF *rho-15* IN *rpsL* or *rpsE* STRAINS, AND *rho-15* METHIONINE AUXOTROPHY IN *rps*+ STRAINS OF *ESCHERICHIA COLI*

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ABSTRACT

The phenotype of *Escherichia coli* K-12 carrying *rho-15* in the genetic background DW319 *ilv lacZ*::IS*1* is described. Seventy-eight percent (70/90) of Ilv+ transductants acquired the following phenotype: temperature-sensitive growth on minimal salts medium, Ts+ growth on complex medium and suppression of the *lac* polar mutation. At 42° on minimal medium, the *rho-15* transductants were cross-fed by a substance diffusing from Rho+ transductants or controls. The requirement for this substance was satisfied by methionine or cystathionine, but not by any other single amino acid or combination of amino acids, by spermidine, or by mono- or divalent cationic salts.——Transduction of *rho-15* into four other Ilv− recipients revealed two phenotypic patterns. Recipients with *rpsL* or *rpsE* ribosomes yielded *rho-15* transductants that were Ts on all media, or Ts on minimal medium whether or not methionine was present. The effect of the ribosome on expression of *rho-15* was confirmed by transduction of appropriate *rps* alleles into DW319, followed by co-transduction of *rho-15* with Ilv+. The growth rate of double *rho-15 rpsL* or *rho-15 rpsE* strains was severely reduced at 42° in comparison with strains carrying any of these single mutations. Models for *rho* and ribosome interaction are presented.

A number of investigators have characterized mutants of *Escherichia coli* affecting the activity of transcription termination factor, rho (DAS, COURT and ADHYA 1976; KORN and YANOFSKY 1976; RICHARDSON, GRIMLEY and LOWERY 1975; SHIGESADA and IMAI 1978). Isolation of such strains is based on selection for cells that can carry on RNA synthesis past a transcriptional stop signal. We have investigated *rho* mutations that suppress the polarity of the *lacZ*::IS*1-319* insertion. When the *rho-15* temperature-sensitive gene is transferred to this genetic background, cells grow at 42° on rich medium and on minimal medium if methionine is present. Hence, the *rho-15* allele does not confer absolute temperature-sensitivity in this genetic background, but rather temperature-sensitive methionine auxotrophy. Absolute temperature sensitivity is conferred by transduction of this strain to streptomycin resistance. Spectinomycin resistance also results in a *rho-15* temperature-sensitive phenotype. These results are inter-

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preted in the context of several models, one considering the effect of ribosomal movement on nascent RNA secondary structure, and the other postulating increased translational fidelity.

MATERIALS AND METHODS

Bacterial strains and media are described in GUTERMAN and HOWITT 1979. Phage P1 was grown as in that reference, except for AD1600 when growth of cells occurred at 34°. Rho phenotype was determined by growth at 42° on MacConkey melibiose agar (MacMel plates). Thiogalactoside transacetylase was assayed as in GUTERMAN and HOWITT (1979). Growth rates of *rps rho-15* strains were determined with freshly isolated clones to avoid contamination by revertants. Table 1 lists the bacterial strains studied.

RESULTS

Phenotype of Ilv+ rho-15 *transductants selected in* DW319 ilv: Phage P1 grown on AD1600 *rho-15* cells was used to transfer the *rho-15* allele into strain DW319 *ilv lacZ*::IS*1-319* by co-transduction with the closely linked marker, *ilv+* . *Ilv+* colonies were selected on minimal media and screened by cloning at 42° on minimal media and LB agar, as well as on MacMel agar. If the *rho-15* allele were to confer the same phenotype on recipient cells as is found in the donor, then a large proportion of the *Ilv+* transductants should be temperature sensitive on rich and minimal media. All Ilv+ clones grew at 42° on rich LB and MacMel agar. On minimal agar, extensive cross-feeding, however, was observed for a large proportion of clones at 42°, *i.e.*, many clones grew if adjacent to Ts+ transductants on one side, but failed to grow if the side of the sector were adjacent to a *ts* transductant. These data suggested that a new requirement for a diffusible compound had been conferred on a large proportion (70 of 90) recipient cells along with the *ilv+* allele.

The 70 clones that were temperature sensitive and subject to being cross-fed on minimal medium at 42° displayed the *rho* mutant phenotype, *i.e.*, red colonies on MacMel agar. The remaining 20 isolates were Ts+ on all media and Rho+, *i.e.*, beige on MacMel agar.

TABLE 1

Bacterial strains, E. coli *K-12*

Strain	Relevant genotype	Source or reference
DW319	*ilv, lacZ-319*::*IS1*, F-, *bgl, rbs, nal*	GUTERMAN and HOWITT 1979
M41	*rho-115* in DW319	GUTERMAN and HOWITT 1979
SA1030	*gal-3, rpsL, his*	DAS *et al.* 1976
AD1600	*gal-3, rpsL, his, rho-15*	DAS *et al.* 1976
GE3, DA431	*rho-15* in DW319 (independent isolates)	This study
SA1615	*gal-3, rps, ilv*, F-, *rpsL*	O. REYES
OR677	*trpE, ilv, his, rpsL*	O. REYES
PB1	F-, *lac* Δ*-74, rbs, ara, nalA, ilv*	J. FELTON
JC5029	*Hfr KL16, rpsE, thr-300, relA, ilv*	B. BACHMAN (CGSC 5230)
LPNA	*lac+, rho+*	GUTERMAN and HOWITT 1979
DR10	from DA431, spontaneous new *rho* allele, Ts+	This study

Conditional methionine requirement: To determine the specific requirement of *rho-15* transductants in the DW319 background, combinations of amino acids and vitamins were placed on cell suspensions on minimal medium, and plates were examined for growth at 42°. Mixtures including methionine, or methionine alone, resulted in a patch of growth of the *rho-15* transductants. The methionine precursor, cystathionine, also supported growth of these strains. No other amino acid or combination would substitute for the methionine requirement. Neither spermidine nor salts of mono- or divalent cations satisfied the methionine requirement.

Thiogalactoside transacetylase confirmation of rho-15 *transductants and derivatives:* One may determine by assay of thiogalactoside transacetylase the extent of transcription past the rho-dependent termination signal of the *lacZ-319*::IS*1* mutation. The quantity of transacetylase is normalized to that in fully induced *lac*+ cell extracts prepared simultaneously in each experiment. The *rho-15* transductants (Table 2: strains DA431, 432, 433) produced twice as much transacetylase (60% of fully induced *lac*+ cells) as cells of the spontaneous mutant M41 *rho-115* (30%) in this background. These data indicate that the *rho-15* mutation results in less *in vivo* function of rho protein than that of the *rho-115* mutation. The *rho-15 in vivo* function does not appear to be temperature sensitive in extracts of one of these mutants (DA431), in that the percent of enzyme found in full induced wild-type cells does not increase between 32° and 42°. However, a Ts+ Rho- partial revertant of *rho-15* (DR10, described below) appears to contain less transacetylase at increased temperatures (from 34.8% at 32° to 25.9% at 42°).

TABLE 2

Thiogalactoside transacetylase levels in rho *mutants and derivatives*

Strain	*lac* allele	*rho* allele	Cell growth temperature	Specific activity*
1. LPNA	+	+	37°	5.30 (100)
DW319	*Z-319*	+	37°	0.108 (2.0)
M41	*Z-319*	*115*	37°	1.57 (29.6)
DA432	*Z-319*	*15*	37°	2.60 (49.1)
DA433	*Z-319*	*15*	37°	3.30 (62.3)
2. LPNA	+	+	37°	4.52 (100)
DA431	*Z-319*	15	37°	2.56 (56.8)
3. LPNA	+	+	32°	4.68 (100)
DA431	*Z-319*	*15*	32°	2.86 (61.1)
DR10	*Z-319*	*15*, Ts+, Met+	32°	1.63 (34.8)
LPNA	+	+	37°	4.92 (100)
DR10	*Z-319*	*15*, Ts+, Met+	37°	1.43 (29.1)
LPNA	+	+	42°	6.25 (100)
DA431	*Z-319*	*15*	42°	3.78 (60.5)
DR10	*Z-319*	*15*, Ts+, Met+	42°	1.62 (25.9)

* Defined as transacetylase activity (A_{412}) divided by protein in extract (mg/ml). Number in parenthesis is the percent of activity of fully induced LPNA *lac*+ *rho*+ cells.

Selection against rho-15 *mutation in DW319 background:* Successive cloning of *ilv rho-15* transductants in the DW319 recipient on MacMel agar revealed that the original, pink (magenta) colonies often generated faster growing, darker red (maroon) colonies. These included clones that are Ts+ on all media. The *rho* mutation in one of these, DR10, is not as severe as in the original isolate (Table 2), as judged by level of transacetylase. It is apparent from the high rate of change of phenotypes of *rho-15* cells that the *rho* allele donated by strain AD1600 confers a significant selective disadvantage.

Methionine auxotrophy is not restricted to DW319 genetic background: Four Ilv⁻ strains in addition to DW319 were used as recipients for transductions with P1 grown on strain AD1600 *rho-15*. Transductants (see Table 3) to Ilv+ were screened for the appearance of methionine auxotrophy at 42°, to determine whether that is attributable to the specific genetic background of DW319 or is a more general phenomenon. Methionine auxotrophy of large proportions of *Ilv*+ transductants were observed in two recipients only: PB1 F⁻ *lac*Δ*-74, rbs, ara, nalA*, and in DW319 F⁻, *rbs, bgl, nal, lacZ*-IS*319*. With three other recipients, SA1615, OR677 and JC5029, however, no relief of temperature sensitivity was observed. *rho* transductants of strain SA1615, which is isogenic to AD1600 *rho-15*, were Ts on rich medium, as is AD1600. A large proportion of Ilv+ transductants of both JC5029 and OR677 were temperature sensitive on minimal media, but Ts+ on rich media. Methionine supplementation did not alleviate the inability of these strains to grow on minimal medium at 42°. Hence, methionine auxotrophy at 42° was found in two of the five *ilv* recipients when *rho-15* was transduced. We conclude that this aspect of the *rho-15* phenotype is not limited to a peculiarity of one particular recipient strain.

Incompatibility of rho-15 *with genes for streptomycin sensitivity or spectinomycin resistance*: The three recipients, SA1615, OR677 and JC5029, in which *rho-15* temperature sensitivity is found on all media or on minimal media but unrelieved by methionine, share a common genetic feature: the ribosomal mutation alleles *rpsL* (formerly *strA*) or *rpsE* (formerly *spcA*). We were interested in this feature since previous attempts to transduce streptomycin resistance from SA1020 to GE3 *rho-15 lacZ*::IS*1-319* failed, *i.e.*, no transductants were

TABLE 3

Phonotype at 42° conferred by rho-15 *on five Ilv⁻ recipients*

Recipient	Sex	Other markers	Growth on media at 42° Complex	Minimal	Min+met
DW319	F⁻	*rbs, bgl, nal, lacZ-319*	+	—	+
JC5029	*Hfr* KL16	*rpsE, thr-300, relA*	+	—	—
OR677	F⁻	*trpE, rpsL, his*	+	—	—
PB1	F⁻	*rbs, ara, nalA, lac*Δ*-74*	+	—	+
SA1615	F⁻	*gal-3, rpsL, his*	—	—	—

* Phenotype at 42° for majority class of Ilv+ transductants.

obtained. Influence of a streptomycin-resistant allele on the *rho* phenotype of *psu-1*, *-2* and *-3* mutations was reported by REYES, GOTTESMAN and ADHYA (1976): *rho* mutant transcription beyond t_L and t_R of λ*N* mutants is decreased in *strA* cells, as reflected in plaque formation, compared to that in cells with a streptomycin-sensitive allele.

To test if *rho-15* conditional lethality is related to *rps* alleles, derivatives of DW319 *ilv lacZ*::IS*1-319* containing *rpsL* from SA1615 or *rpsE* from JC5029 were constructed. These were then transduced to Ilv$^+$ with AD1600 as the donor to introduce *rho-15*. The rationale for this procedure was to avoid direct selection of a *rho* or ribosmal mutation in the presence of the other, since previous data indicated inability to select *rpsL* directly in *rho-15* cells. In fact, Ilv$^+$ transductants were obtained, and fell into two classes: large colonies after two days of growth on minimal glucose media at 32°, and tiny colonies after three to five days.

Growth rates at 32° and 42° of strains containing combinations of *rho*$^+$ or *rho-15*, and *rps*$^+$, *rpsL* or *rpsE* are shown in Table 4. It is apparent from these data that the combination of *rho-15* and a ribosomal mutation confers severe inability to grow at 42° (doubling time extrapolated to 450 min). Growth at 32° also is retarded in *rho-15* ribosomal double mutants: *rho-15 rpsL* cells take 120 minutes to double compared to 60 minutes for rho$^+$ *rpsL* cells, 107 minutes for *rho-15 rps*$^+$, and 57 minutes for *rho*$^+$ *rps*$^+$. The growth-curve data confirm the hypothesis that the combination of the *rho-15* allele with at least certain ribosomal mutations is the cause of the temperature-sensitive phenotype.

Transacetylase was not assayed in strains containing both *rho-15* and an *rps* mutation since the extremely slow growth rate of the cells resulted in high rate of reversion and accumulation of suppressing alleles. Rapid growing derivatives of the *rho-15 rpsE* strains included *rho*$^+$, as well as spectinomycin-sensitive classes. The majority of rapidly growing derivatives of *rho-15 rpsL* cells were *rho*$^+$ (beige on MacMel) or *rho* mutants giving different colony color on MacMel medium (pale pink). No *rspL*$^+$ streptomycin-sensitive derivatives were obtained,

TABLE 4

Growth rates of rho-15, *and double* rho-15 rpsL *and* rho-15 rpsE *strains in glucose minimal medium*

rho genotype	Ribosome genotype	Doubling time, min*	
		32°	*42°*
+	+	57	39
+	*rpsL*	60	50
+	*rpsE*	114	68
rho-15	+	107	96
rho-15	*rpsL*	120	(450)†
rho-15	*rpsE*	159	(450)†

* A culture of cells grown at 32° in OM glucose medium was divided, and growth continued at 32° and 42°, monitored spectrophotometrically at 600 nm.
† Extrapolated.

perhaps due to nonrevertibility of the *rpsL* allele. The high frequency of reversion of double mutant *rho-15 rpsE* cells to *rho*$^+$ or to *rpsE*$^+$ demonstrates the nonviability of cells carrying mutations in both systems.

DISCUSSION

We show here that the phenotype resulting from the *rho-15* allele is influenced by other aspects of the genetic background of the cell, particularly the ribosomal alleles. In cells with wild-type ribosomes, the *rho-15* mutation produces partial methionine auxotrophy at 42°. In cells with streptomycin-resistant (*rpsL*) ribosomes, the *rho-15* mutation causes severe limitation of growth at 42° regardless of the medium.

rho mutations have been shown to result in elongation of RNA beyond a rho-dependent attenuator into the genes for the biosynthetic enzymes in the *trp* operon, altering the regulation of these enzymes (KORN and YANOFSKY 1976). Regulation by such attenuation has been observed also in the *phe* operon of *E. coli* (ZURAWSKI *et al.* 1978) and the *his* operons of *E. coli* (DINOCERA *et al.* 1978) and *Salmonella typhimurium* (BARNES 1978). If attenuation regulated methionine biosynthesis, one would predict that cells with the *rho* phenotype should overproduce methionine, whereas we find here that *rho-15* results in methionine auxotrophy. This suggests that attenuation is not involved in methionine biosynthesis, so that some other mechanism must be invoked. The *rho-15* methionine auxotrophy may result from a large depletion of cell methionine pools, for example, if *rho* mutants overproduced some methylating function that was regulated by attenuation.

It may be relevant that the first enzyme of methionine biosynthesis, homoserine transsuccinylase, is inherently temperature sensitive (RON and DAVIS 1971). If this enzyme were synthesized in less than sufficient quantity and were out of balance with other cell proteins, then a temperature shift might cause significant disability in the cellular methionine biosynthetic capacity.

More interesting is the incompatibility of the *rho-15* mutation with ribosomal mutations. A number of mechanisms are suggested. Since ribosomal mutations such as *rpsL* increase the fidelity of translation, the combination of *rpsL* with *rho-15* may reduce the mistranslation of the *rho-15* mutation. In this model, the strict *rho-15* protein species would cause absolute temperature sensitivity, and growth of *rho-15* cells at 42° could be attributed to translational errors that would allow synthesis of a minority of rho protein molecules with increased function.

A second model is concerned with speed of ribosomal movement and secondary structures in nascent RNA. YANOFSKY and his coworkers (LEE and YANOFSKY 1977) have suggested that the relative stability of two alternative stem-and-loop structures in the nascent *trpL* RNA segment can influence the extent of termination. The *rpsE* and *rpsL* mutations directly affect proteins S5 and S12, respectively, of the 30s subunit of the ribosome. It is possible that, with these alleles, attenuation of many amino acid biosynthetic operons is eliminated, leading to overproduction of enzymes and unbalanced growth. *rpsL* mutations

result in ribosomes that move more slowly along the mRNA than wild-type ribosomes (GALAS and BRANSCOMB 1976). The rate of flux of ribosomes might influence the balance between 5′ proximal and 5′ distal stem-and-loop structures, such that slow-moving ribosomes favor 5′ proximal structures and elongation of RNA, and rapidly-moving ribosomes similarly favor 5′ distal structures and termination. In support of this model is the finding that streptomycin resistance in *rho*$^+$ cells results in anti-polarity, *i.e.*, increased distal gene product in several operons (A. ULLMANN, personal communication). If this model is correct, then the combination of loss of termination due both to slowness of ribosomes and to *rho* mutation may result in the increased lethality that we observe here.

The major significance of the two models lies in the relative importance assigned by each to rho protein as an essential function. If the model of ribosomal fidelity is correct, then wild-type rho is essential to the cell, presumably as a transcription termination factor. Several investigators have demonstrated elegantly that *rho* amber mutations are lethal in the absence of a suppressing tRNA allele (GUARENTE and BECKWITH 1978). However this experiment was performed in a streptomycin-resistant strain specifically to avoid ribosomal ambiguity. A test of the prediction that translational ambiguity causes the Ts$^+$ phenotype that we observe in *rho-15* strains is to analyze the number of species of rho protein in *rho-15* cells, in *rho-15 rps* and in wild-type cells. However, wild-type or suppressed rho may be present as a minority species, so that failure to obtain a positive result would not rule out the translational fidelity model.

Ultimately, at the molecular level it is the speed of the ribosome determined by the *rpsL* allele that affects transitional fidelity and may influence RNA secondary structure. Hence the two models may be difficult to distinguish. The significance of these results lies in construction of strains with *rho* alleles, and in their stability. It is possible, for example, that the more wild-type phenotype of *psu* mutants carrying an *rpsL* allele (REYES, GOTTESMAN and ADHYA 1976) is due to selection of clones with weaker *rho* mutations than in the original strain. *rho* mutations may be sufficiently lethal so that selection of cells with altered phenotypes occurs at high frequency, necessitating monitoring of strains.

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