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Reduced Transposition in *rho* Mutants of *Escherichia coli* K-12

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Substantially reduced frequencies of transposition for the transposons Tn*5* and Tn*9* and the insertion sequences IS*1* and IS*5* were observed in several *rho* mutants of *Escherichia coli* K-12 compared with those observed in their isogenic wild-type counterparts. The lower transposition frequencies could be due to decreased supercoiling of DNA, to altered expression of required genes, or to aberrant transcription of transposon or target DNA resulting from the lack of transcription termination at Rho-sensitive sites in *rho* mutants.

Transposable elements in *Escherichia coli* require both bacterial and transposon-encoded proteins for their transposition. From mutational studies, several host genes involved in transposition have been identified: *polA* (4, 27, 31), encoding DNA polymerase I; *gyrA* and *gyrB* (14, 17), encoding DNA gyrase; *topA* (30), encoding DNA topoisomerase I (but see reference 17); and *tnm* and *ger* (29). We report here that *rho* mutants of *E. coli* which were defective in transcription termination were markedly deficient in the transposition of the transposons Tn*5* and Tn*9* and the insertion sequences IS*1* and IS*5*.

The *rho* gene product of *E. coli* K-12 is required for transcription termination at Rho-sensitive sequences (9, 24). Bacteria carrying the *rho-15*(Ts) mutation make a truncated Rho protein because of the presence of an IS*1* in the *rho* structural gene (15). The Rho protein from *rho-15*(Ts) and other *rho*(Ts) mutants does not carry out transcription termination or the coupled ATPase reaction in vitro at temperatures ranging from 32 to 50°C (5). However, Rho protein from *rho-15*(Ts) cells has a temperature-sensitive poly(C)-dependent ATPase activity, and the viability of these cells is similarly temperature sensitive (5). The experiments described here were done at 31°C, at which temperature the *rho*(Ts) mutants were viable, though termination is defective (except that thermal induction of λ *c*I857 and Mu *c*ts62 lysogens involved incubation at 40°C for 15 min).

(A preliminary report of this work was presented at the Sixth Mid-Atlantic Extrachromosomal Genetic Elements Meeting, 1982 [J. L. Rosner and A. R. Datta, Plasmid **10**:207, 1983].)

Decreased transposition in *rho* mutants was first indicated by the finding that β-glucoside-utilizing (Bgl^+) variants were at least 200-fold less frequent in *rho*-15(Ts) mutants than in wild-type cells (Table 1). Wild-type and mutant bacterial cultures grown overnight at 31°C in L broth medium were diluted and then spread and incubated on indicator plates with a β-glucoside (MacConkey agar plates with 1% salicin). After 3 days at 31°C, Bgl^+ variants appeared as papillae on the colonies, and the papillae in each of approximately 100 well-separated colonies were counted by using a dissecting microscope. Bgl^+ variants arise most frequently because of transposition of an endogenous IS*1* or IS*5* (5 to 10 copies of each are normally present on the *E. coli* K-12 chromosome [22, 28] to the cryptic *bgl* operon (23). The absence of Bgl^+ papillae in *rho*-15(Ts) bacteria, as determined by the method described above, suggested two possibilities: either *rho-15*(Ts) strains are impaired in carrying out both IS*1* and IS*5* transpositions or they are defective in the expression of the *bgl* operon even after it has been activated by transposition.

To distinguish between these alternatives, Bgl^+ variants were isolated from a *galP3*::IS*2 ilv rho*$^+$ strain. The *rho-15*(Ts) allele was then introduced into these strains from *rho-15*(Ts) *ilv*$^+$ bacteria by P1 *vir*-mediated cotransduction with *ilv*. Strains carrying the *rho-15*(Ts) allele were identified by their temperature-sensitive growth and by their Gal^+ phenotype, which is due to readthrough transcription of *galP3*::IS*2*. These *rho-15*(Ts) transductants remained Bgl^+. Thus, *rho*$^+$ is not required for the expression of the Bgl^+ phenotype, suggesting that it is needed only for the transposition event that activates the operon.

Decreased frequencies of Bgl^+ variants were also noted with other *rho* mutants. These frequencies, estimated from the number of Bgl^+ papillae found per colony, were 0.05, 0.5, and 4% of that for wild-type bacteria for *rho-15*(Ts), *rho-12*(Ts) (7), and *rho-955*(Ts) cells (obtained from O. Reyes), respectively (Table 1). These results correlate roughly with the abilities of these mutants to suppress the *galP3*::IS*2* mutation, a measure of the inactivity of Rho in these strains. *rho-15*(Ts) and *rho:112*(Ts) appear to be fully Gal^+, while *rho-955*(Ts) is only faintly Gal^+ on galactose indicator plates.

rho$^+$ was at least partly dominant over *rho-15*(Ts) with regard to the Bgl^+ papillation assay. When a *rho-15*(Ts) strain was transformed with pEG25, a derivative of the multicopy plasmid pBR322 that carries the *rho*$^+$ gene (obtained from E. Gulletta), Bgl^+ papillation was normal. When the *rho-15*(Ts) mutant was lysogenized with the specialized transducing phage λ *rho*$^+$524 (15), papillation was increased, but not to full wild-type levels. These results suggest that a high copy number of *rho*$^+$ genes may be necessary for complete dominance over *rho-15*(Ts) in the Bgl^+ papillation assay. We also found that a full revertant of *rho-15*(Ts), strain EG1641 (15), showed normal papillation, while a partially thermoresistant revertant, strain EG1631 (15), did not. We conclude that the defect in transposition is due to the *rho-15*(Ts) mutation and that this defect can be complemented by the wild-type gene.

The decreased frequency of *bgl* activation could be due to an overall reduction of transposition frequencies in *rho* mutants or to an inability of IS*1* and IS*5* to transpose to the

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TABLE 1. Effect of *rho* alleles on *galP3* suppression, salicin papillation, and transposition of Tn*5* and Tn*9* from chromosome to λ prophage

rho allele (strain no.)	Color on MacConkey-galactose[a]	No. of Bgl$^+$ papillae per colony[b]	No. of transductants per total phage[c]	
			Cam^r (Tn*9*)	Kan^r (Tn*5*)
+ (N6405)	White	20–40	4.5×10^{-6} (1.0)	1.3×10^{-5} (1.0)
955(Ts) (N6406)	Weak red	0.7	4.7×10^{-7} (0.10)	5.6×10^{-7} (0.043)
15(Ts) (N6407)	Red	<0.01	1.9×10^{-7} (0.042)	1.7×10^{-7} (0.013)
112(Ts) (N6408)	Red	0.1	3.6×10^{-7} (0.080)	3.3×10^{-7} (0.025)

[a] Overnight cultures grown at 31°C in L broth medium were streaked on MacConkey agar supplemented with 1% galactose and incubated at 31°C for 2 to 4 days.
[b] The distribution of papillae per colony conformed to the Poisson distribution, validating the use of the average.
[c] Each value is the average of the yield from 5 to 8 independent lysates. Values in parentheses are normalized to 1.0 for *rho*$^+$.

specific sites required for activation of *bgl*. To test this possibility, we measured the transposition of Tn*9*, an IS*1*-bracketed transposon, and of Tn*5*, a nonrelated transposon, from sites on the chromosome to a λ *c*I857 *b*515 *b*519 prophage in wild-type bacteria and *rho*(Ts) mutants. Chromosomal insertions of Tn*5* and Tn*9* were isolated in the Ilv$^-$ Rho$^+$ *galP3*::IS*2* strain AD1615 (from S. Adhya) by infection with appropriate λ::Tn phage (8) and then lysogenized with λ *c*I857 *b*515 *b*519. Derivatives carrying *rho*,$^+$ *rho-955*(Ts), *rho-15*(Ts), or *rho-112*(Ts) alleles (N6405 through N6408, respectively) were then prepared by cotransduction with *ilv*$^+$ by using P1 *vir* lysates grown on the appropriate *ilv*$^+$ *rho* donor strains. The strains were grown in LB medium to 10^8 cells per ml, shifted to 40°C for 15 min, and then returned to 31°C for an additional 105 min. The lysates were treated with $CHCl_3$ and clarified by centrifugation, and the titer of phage (about 10^{10}/ml for each strain) was determined with strain YMelC as an indicator. Samples containing 10^8 phage were then incubated with 2×10^8 YMelC cells at 31°C for 30 min and plated on L broth agar supplemented with 25 mg of chloramphenicol or 20 mg of kanamycin sulfate per liter to determine the number of λ::Tn*9* or λ::Tn*5* transducing particles. Our results showed that Tn*9* transposition frequencies in the mutants were 10 to 20 times lower than those for wild-type bacteria, and Tn*5* transposition frequencies in the mutants were 20 to 70 times lower (Table 1).

Similar results were obtained for the transposition of Tn*5* from sites in *thr*, *arg*, and *lys* genes to a λ prophage in otherwise isogenic *rho*$^+$ and *rho-15*(Ts) strains (Table 2). P1 *vir* lysates (prepared by M. Guyer) of the Tn*5*-induced auxotrophs Arg$^-$, Lys$^-$, and Thr$^-$ (isolated by H. I. Miller) were used to transduce AD1615 (λ *c*I857 *b*515 *b*519) to kanamycin-resistant auxotrophy. *rho*$^+$ and *rho-15*(Ts) derivatives of these strains were then isolated by cotransduction with *ilv*, and lysates of these strains were prepared and assayed for λ::Tn*5* transducing phage, as described above. The phage yields from the *rho-15*(Ts) strains were about 5 to 10 times lower than the yields from the corresponding *rho*$^+$ strains. The frequencies of λ::Tn*5* transducing phage per total phage were 10 to 50 times lower in *rho-15*(Ts) lysates than in *rho*$^+$ lysates. Thus, *rho* mutants were impaired in carrying out IS*1*, IS*5*, Tn*5*, and Tn*9* transpositions.

TABLE 2. Influence of *rho* on Tn*5* transposition to λ prophage from several chromosomal sites

Tn*5*-induced auxotroph	No. of Kan^r transductants per phage[a]		Ratio, *rho-15*(Ts)/*rho*$^+$
	rho$^+$	*rho-15*(Ts)	
Arg$^-$	5.2×10^{-5}	1.7×10^{-6}	0.032
Lys$^-$	6.3×10^{-5}	6.0×10^{-6}	0.095
Thr$^-$	2.9×10^{-5}	6.1×10^{-7}	0.021

[a] Each value represents the average of the yield from 8 to 11 independent lysates.

Bacteriophage Mu uses a replicative mode of transposition for lytic growth (2) and a mostly nonreplicative mode of transposition to lysogenize *E. coli* (16, 18, 21). It was previously reported that Mu grows poorly after infecting *rho* mutants (6). We found that this inability to produce plaque-forming phage was also true of thermally induced Mu *c*ts62 lysogens carrying *rho* mutations. Whereas *rho*$^+$ lysogens produced about 260 PFU per cell, *rho-955*(Ts) and *rho-15*(Ts) lysogens produced only 0.001 PFU per cell, and *rho-112*(Ts) lysogens produced about 6 PFU per cell. This defect in phage production after thermal induction of *rho* mutants could be due to a defect in the Mu lytic transposition process or in some other lytic process. We next examined lysogenization by a Mu *c*ts::Amp phage in *rho*$^+$ and *rho-15*(Ts) cells at 31°C. Cells were infected at multiplicities ranging from 0.1 to 10 phage per cell, and the number of ampicillin-resistant lysogens was determined. Throughout the range of multiplicities used, the frequency of lysogens per infected cell was about 5×10^{-3} for *rho*$^+$ cells and 4×10^{-2} for *rho-15*(Ts) cells. This eightfold-greater lysogenization frequency in *rho-15*(Ts) cells may reflect poorer lytic growth of Mu rather than more efficient lysogenization.

While precise excision is not directly related to transposition (10, 13), it was of interest to measure such events in *rho* mutants. When otherwise isogenic strains with Tn*5* inserted in a *gua* or a *lys* locus were tested, no significant differences in reversion to prototrophy were observed. There were approximately 10^{-7} Gua$^+$ revertants per cell per generation and approximately 10^{-8} Lys$^+$ revertants per cell per generation. Thus, precise excision of Tn*5* was similar in *rho*$^+$ and *rho-15*(Ts) strains.

rho mutations have extremely pleiotropic effects (6), presumably because abnormal transcription termination affects the expression of many genes. Thus, it is quite possible that these termination defects cause reduced (or excessive) synthesis of some transposon or bacterial gene product(s) involved in transposition. Fassler et al. (11, 12) have recently shown that *rho-15*(Ts) mutants are defective in DNA supercoiling. This defect could be due either to a direct role for Rho protein in supercoiling or to an indirect role in regulating the levels of DNA gyrase or topoisomerase I. Since reduced supercoiling has been shown to reduce the transposition of Tn*5* (17) and the lytic (replicative) transposition of Mu (21, 25), reduced supercoiling in *rho* mutants could account for the reduced transpositions and lytic growth of Mu seen here. With respect to Mu transposition, *rho* mutants bear resemblance to *himA* mutants which lack the α subunit of integration host factor. *himA* mutants are lysogenized by Mu at nearly normal frequencies but produce drastically reduced bursts of Mu phage after infection (20).

The efficient lysogenization by Mu of the *rho-15*(Ts) mutant could reflect a lesser requirement of simple (integrative) transposition for supercoiling. Mu integrative transposition in vitro was less sensitive than replicative transposition to a DNA gyrase inhibitor (21).

Two other possibilities for the effect of *rho* on transposition may be relevant. First, readthrough transcription of transposon DNA in *rho* mutants could interfere with the transposition event itself. Recent experiments have shown that transcription of an IS*1* or IS*50* from an upstream promoter reduces the transpositional activity of the element (1, 3, 19, 26). Second, altered transcription of potential target sites in *rho* mutants may lower their suitability for transposition.

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