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A Novel *rho* Promoter::Tn*10* Mutation Suppresses an *ftsQ1*(Ts) Missense Mutation in an Essential *Escherichia coli* Cell Division Gene by a Mechanism Not Involving Polarity Suppression

DOUGLAS R. STORTS† AND ALVIN MARKOVITZ*

Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois 60637

Received 14 June 1990/Accepted 12 October 1990

An extragenic suppressor of the *Escherichia coli* cell division gene *ftsQ1*(Ts) was isolated. The suppressor is a Tn*10* insertion into the −35 promoter consensus sequence of the *rho* gene, designated *rho* promoter::Tn*10*. The *ftsQ1*(Ts) mutation was also suppressed by the *rho-4* mutant allele. The *rho* promoter::Tn*10* strain does not exhibit *rho* mutant polarity suppressor phenotypes. In addition, overexpression of the *ftsQ1*(Ts) mutation does not reverse temperature sensitivity. Furthermore, DNA sequence analysis of the *ftsQ1*(Ts) allele revealed that the salt-remediable, temperature-sensitive phenotype arose from a single missense mutation. The most striking phenotype of the *rho* promoter::Tn*10* mutant strain is an increase in the level of negative supercoiling. On the basis of these observations, we conclude that the *ftsQ1*(Ts) mutation may be suppressed by a change in supercoiling.

A large number of genes have been implicated in *Escherichia coli* cell division. Most of the characterized genes are clustered around the 2- and 76-min regions of the *E. coli* chromosome (3; for reviews, see references 8 and 9). The 2-min region has been extensively studied and includes the *ftsQ*, *ftsA*, *ftsZ* (*sulB*, *sfiB*), and *ftsI* (*sep*, *pbpB*) genes. This region has been sequenced (33, 35, 36, 47, 48), and the individual protein products have been characterized (8, 9, 39, 48). With the exception of the *ftsI* gene product (8, 9), little is known about the function of the individual components. Ward and Lutkenhaus (44) demonstrated that overexpression of *ftsZ* results in minicell formation. This observation and the demonstration that FtsZ is the target of the cell division inhibitor SulA (8, 9) have led to the conclusion that FtsZ may be a rate-limiting component in cell division.

The *ftsQ* gene product is an essential component of *E. coli* cell division. Conditional lethal mutations have been isolated that filament and die at restrictive temperatures on medium without salt (4). We recently demonstrated that the *ftsQ* gene encodes a stable, inner membrane-associated protein of 31,400 Da that is expressed at low levels in the cell (39). However, overproduction of the FtsQ protein had no apparent affect upon cellular morphology in wild-type cells such as strain AMC290 (Table 1).

To further investigate the role of FtsQ in cell division and to examine potential interactions with other cell division proteins, we isolated an extragenic suppressor of *ftsQ1*(Ts). In this report, we characterize the *ftsQ1*(Ts) mutation and demonstrate that mutations in either the *rho* gene or its promoter are capable of suppressing the *ftsQ1*(Ts) mutation.

MATERIALS AND METHODS

Media, bacterial strains, and bacteriophages. The bacterial strains and phages used in this study are listed in Table 1. P1 transductions were performed as described previously (30). Bacteria were routinely grown in complex medium (LB [29]) or in M9 minimal medium (30) without $CaCl_2$ supplemented with amino acids (50 μg/ml) as necessary. LB medium lacking NaCl (TEY) was used to determine the temperature sensitivity of the *ftsQ1*(Ts) mutant strains. Kanamycin and carbenicillin were each used at a concentration of 50 μg/ml for agar media and 25 μg/ml for liquid media; tetracycline hydrochloride was used at a concentration of 10 μg/ml. MacConkey agar–galactose plates (Difco) contained 1% galactose. Rifampin sensitivity was determined on LB plates containing 5 or 10 μg of rifampin per ml (13). Anthranilate sensitivity (31, 32) was determined on M9 minimal plates containing leucine (50 μg/ml) and anthranilate (100 μg/ml). Streptomycin sulfate was used at a concentration of 200 μg/ml.

Mapping techniques. Tn*10* transposition into the chromosome from λ1098 was performed as recommended by Way et al. (45). This phage contains a defective Tn*10* that is only capable of a single transposition event. For initial mapping of Tn*10* in strain AMC501, a total of 17 Hfr strains (including strain PK3Hfr) were transduced to tetracycline resistance with P1 grown on AMC501. The tetracycline-resistant Hfr donor strains were then conjugated with AMC436 as recommended by Miller (30). After 10 and 90 min, tetracycline-resistant recipients were selected on LB containing tetracycline hydrochloride and streptomycin. A time-of-entry experiment was performed by using PK3 Hfr::Tn*10* as the donor and AMC436 as the recipient. Aliquots of the mixed culture were disrupted at 1-min intervals and plated as described above. More precise mapping was performed using P1-mediated generalized transduction.

Isolation of extragenic suppressors of *ftsQ1*(Ts). Strain AMC436 *ftsQ1*(Ts) was plated on TEY at 42°C at 10^6 to 10^7 cells per plate. Plates treated with UV radiation yielded discrete colonies that were picked, whereas unirradiated plates displayed a light haze all over the plate, but there were no obvious large colonies. Ten purified, isolated clones were transduced to tetracycline resistance with P1 grown on strain AMC416 [*leu*::Tn*10* *ftsQ1*(Ts)]. The *leu*::Tn*10* marker is 70% cotransduced with *ftsQ1*(Ts). The tetracycline-resistant transductants were tested for temperature sensitivity by streaking on TEY at 41°C. If the original colonies arose because of a reversion mutation at the *ftsQ* locus, a temper-

* Corresponding author.
† Present address: Ambion, Inc., Austin, TX 78744.

TABLE 1. Bacterial strains and phages

Strain or phage	Relevant genotype and phenotype	Source (reference)
E. coli K-12		
DH5α	*recA1* φ80Δ*lacZ*Δ*M15*	BRL[a]
JM101	Δ(*lac-pro*) *thi supE*(F′ *traD36 proAB*$^+$ *lacI*^{q} *ZDM15*)	BRL (29)
Q359	*hsdR*(Kr$^-$ Km$^+$) *supE* φ80 (P2)	
NK5012	C600 *thr leu supE tonB* (T1^r, 15^r, φ80^r)	N. Kleckner
SG13022	F′ *his pyrD lon-100 leu*::Tn*10 strA thi*	S. Gottesman
TOE-1	*ftsQ1*(Ts)	K. J. Begg (4)
AB 259	Hfr *thi-1 rel-1* λ$^-$	E. A. Adelberg (25)
MC169	*proC*$^+$ *capR9 galU*	This laboratory (49)
AMC290	*proC capR*$^+$ *leu*$^+$ *ftsQ*$^+$ *sup*^{0} TEY$^+$	This laboratory (39)
AMC405	*ftsQ1*(Ts) *leu*::Tn*10* TEY$^-$[b]	P1(SG13022) × TOE-1
AMC416	*proC capR*$^+$ *ftsQ1*(Ts) *leu*::Tn*10 sup*^{0} TEY$^-$	P1(AMC405) × AMC290
AMC424	*proC capR*$^+$ *ftsQ*$^+$ *ftsA*$^+$ *leu*$^+$ *sup*^{0} TEY$^+$	P1(AB 259) × AMC413
AMC426	*proC capR*$^+$ *ftsQ1*(Ts) *leu*$^+$ *sup*^{0} TEY$^-$	P1(AB 259) × AMC416
AMC434	*proC*$^+$ *capR*$^+$ *ftsQ*$^+$ *leu*$^+$ *sup*^{0} TEY$^+$	P1(MC169) × AMC424
AMC436	*proC*$^+$ *capR*$^+$ *ftsQ1*(Ts) *leu*$^+$ *sup*^{0} TEY$^-$	P1(MC169) × AMC426
AMC436-64	*ftsQ1*(Ts) *suqA* TEY$^+$	UV-induced revertant of AMC436
AMC501	*leu*$^+$ *ftsQ1*(Ts) *rho* promoter::Tn*10* TEY$^+$	This study
AMC502	*leu*$^+$ *ftsQ*$^+$ Tn*10* TEY$^+$	This study
AMC503	*leu*$^+$ *ftsQ*$^+$ Tn*10* TEY$^+$	This study
DSC23	*leu*$^+$ *ftsQ1*(Ts) *ilvY*::Tn*10* TEY$^-$	P1(IT1022) × AMC436
DSC24	*leu*$^+$ *ftsQ1*(Ts) *ilvY*$^+$ *rho-4* TEY$^+$	P1(Morse 2055) × DSC23
DSC25	*leu*$^+$ *ftsQ*$^+$ *rho* promoter::Tn*10* TEY$^+$	P1(AMC501) × AMC434
IT1022	F$^-$ *his-871 relA1 rpsL181 gal-3 ilvY864*::Tn*10*	Tessman (42)
Morse 2034	F$^-$ *trpE9851 leu-277* λ$^-$ IN(*rrnD-rrnE*)1	B. Bachmann
Morse 2055	F$^-$ *trpE9851 leu-277 rho-4* λ$^-$ IN(*rrnD-rrnE*)1	B. Bachmann (31)
JP58	F$^-$ *thi-1 ilvC7 argE3 galK2 mtl-1 xyl-5 strA704 tfr-3*? λ$^-$ *supE44*?	B. Bachmann
PK3	Hfr$_v$ *thr-1 leuB6 thi-1 lacY1 azi-15 tonA21 supE44*	B. Bachmann
E177	F$^-$ *thr-1 leuB6 thi-1 thyA6 deoC1 dnaA177 lacY1 strA67 tonA21* λ *supE44*	B. Bachmann
RE74	Hfr *tonA22 relA1 pyrE41 uhp-40 gltSo14 tna-6 metB1* T2^r	B. Bachmann
JF448	F$^+$ *asnB32 relA1 spoT1 bglR13 asnA31 rbs-4 thi-1*	B. Bachmann
AB2545	Hfr *relA1 spoT1 metE46 thi-1* λ$^-$	B. Bachmann
Phage		
P1 *vir*		Laboratory stock
M13mp19		BRL
T7		Laboratory stock
λ1098	Contains a defective Tn*10*	N. Kleckner (45)
λEMBL3		Stratagene (12)
λDSC100	Contains the *rho* promoter::Tn*10* allele from AMC501	This study
λDSC104	Contains the *ftsQ1*(Ts) allele from AMC436	This study
λ *c*I857		D. Kaiser
λ *c*I857 *N7*		A. Campbell via W. Epstein
λ cI857 N7 N53		A. Campbell via W. Epstein
λ cI857 N7 N53 nin5		D. Court

[a] BRL, Bethesda Research Laboratories.
[b] TEY$^-$, Inability to grow on LB medium without NaCl at 41°C.

ature-sensitive phenotype would cotransduce at a 70% frequency. Because our primary goal was to obtain extragenic suppressors, we discarded the eight isolates that were transduced to temperature sensitivity. Two of the 10 temperature-resistant isolates were not transduced to temperature sensitivity on introduction of the *ftsQ1*(Ts) allele and thus harbored a suppressor(s) of *ftsQ1*(Ts) that failed to map at the 2-min region of the *E. coli* chromosome. One of the two, strain AMC436-64 [designated *ftsQ1*(Ts) *suqA*], was selected for further characterization.

Isolation of recombinant λ phages. Chromosomal DNA isolated from AMC436 and AMC501 was partially digested with *Sau*3A and cloned into *Bam*HI-digested EMBL3 phage DNA as described by Frischauf et al. (12). The phage DNA was packaged in vitro by using a Gigapack-Plus kit obtained from Stratagene. Packaged phage was plated on *E. coli* Q359 to select for recombinant phage. Plaque transfers onto nitrocellulose and hybridization conditions were as recommended by the supplier (Schleicher & Schuell, Inc.). The AMC436 library was screened with a 970-bp *Eco*RI-PvuII fragment, which was isolated from pDSC73 (39) and labeled with [α-^{32}P]dCTP by using an oligolabeling kit (Pharmacia LKB) as specified by the supplier. This 970-bp fragment contains all of the *ftsQ* gene as well as the 5′ end of *ftsA*. One of the hybridizing phages was designated λDSC104. The AMC501 library was screened with [α-^{32}P]dCTP-labeled pNK82 (14). This hybridization probe contains Tn*10* sequences from the IS*10R* to the *Eco*RI site within the *tetA* gene. One of the hybridizing phages was designated λDSC100.

Plasmid constructions. The plasmids described in this report are shown in Fig. 1. Plasmids pDSC73 and pDSC78 have been described previously (39). Plasmid pDSC75 contains a 2,470-bp *Xho*I-*Hin*dIII fragment isolated from

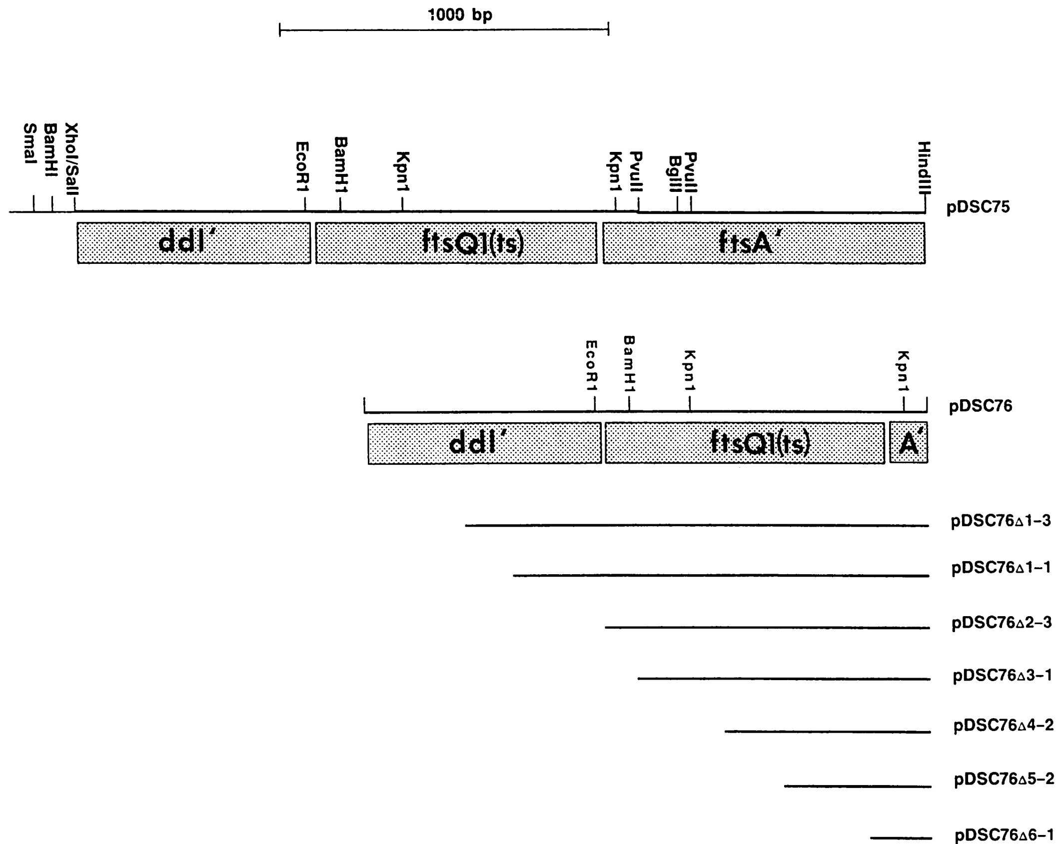

FIG. 1. Schematic diagrams of plasmids used in this study.

λDSC104 cloned into *Sal*I-*Hin*dIII-digested pT7T3-19U (Pharmacia LKB). This 2,470-bp fragment contains most of the *ddl* gene, all of *ftsQ1*(Ts), and a large portion of *ftsA*. Plasmid pDSC76 was constructed by digesting pDSC75 with *Sma*I and *Pvu*II and cloning the 1,640-bp fragment into *Hin*cII-digested pT7T3-19U. Unidirectional deletions were constructed in plasmid pDSC76 by using the exonuclease III protocol described by Henikoff (15).

Isolation of DNA. Bacterial strains were transformed by the $CaCl_2$ method (22). Plasmid DNA was isolated from minipreparations of transformed DH5α (16). Large-scale isolation of plasmid DNA from DH5α was performed as described by Holmes and Quigley (17). Lambda phage DNA was obtained as described by Maniatis et al. (26). Chromosomal DNA was isolated as previously described (2).

DNA sequencing. Double-stranded (plasmid) and single-stranded (M13) DNA sequencing was performed with modified T7 DNA polymerase (Sequenase; United States Biochemical) as recommended by the supplier. Plasmid pDSC76 and deletion derivatives of pDSC76 were sequenced with a standard M13 (−40) primer. The second strand was sequenced with a standard T7 sequencing primer or with synthetic oligonucleotides (Table 2). The λDSC100 phage was partially digested with *Sau*3A, and the fragments were subcloned into M13mp19. The M13mp19 clones were screened with oligonucleotides complementary to the outside end of IS*10R* inverted repeats bracketing the Tn*10* (Table 2). Two of these hybridizing, recombinant M13 phage were isolated and sequenced by using a standard M13 (−40) primer and the synthetic oligonucleotides complementary to the outside end of IS*10R*.

Measurement of plasmid supercoiling. Plasmid DNA was isolated from fresh overnight cultures of pBR322-transformed AMC434, AMC436, AMC436-64, AMC501, and DSC25 by using the protocol of Lodge et al. (24). The samples were electrophoresed in a 1% agarose gel containing 12 μg of chloroquine per ml for 16 h at 3 V/cm. After the gel was washed for 4 h with double-distilled H_2O, the samples were capillary blotted onto GeneScreen (New England Nuclear Corp.) as recommended by the manufacturer. The DNA was hybridized with [α-^{32}P]dCTP random labeled pBR322 and washed as recommended by New England Nuclear.

RESULTS AND DISCUSSION

Characterization of the *ftsQ1*(Ts) mutation. The region extending from the *Xho*I site near the amino terminus of *ddl*

TABLE 2. Oligonucleotides used for DNA sequencing and hybridization

Complementary sequence to gene	Oligonucleotide sequence	Reference
ftsQ	5′ AACTGCCAGGAACGCCG 3′	35
ftsQ	5′ CGTTCTGGCGGCACGCT 3′	35
ftsQ	5′ AATGGCGTTCACCGGTC 3′	35
ddl	5′ TACCTGCCTGACGTGCC	36
ddl	5′ GGTTCCGGACGGTTGAA 3′	36
IS*10R*	5′ CTGATGAATCCCCTAATGATTTT 3′	14
IS*10R*	5′ AAAATCATTAGGGGATTCATCAG 3′	14

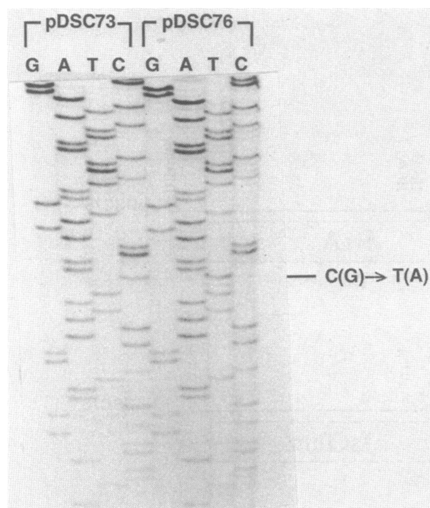

FIG. 2. Comparison of the DNA sequences of *ftsQ*$^+$ (pDSC73) and *ftsQ1*(Ts) (pDSC76). The point mutation is indicated by an arrow.

to the *Pvu*II site within *ftsA* was subcloned from pDSC75 into pT7T3-19U, as described above (Fig. 1). Because this was a blunt-end cloning, we expected to obtain clones with the insert in either orientation. All 12 clones examined were oriented with the truncated *ddl* gene adjacent to the T3 promoter. We did not ascertain the reason all of the clones had this orientation, but we assume that transcription from the lactose promoter is deleterious when transcribed in the sense orientation. A series of *Exo*III-generated nested deletions (Fig. 1) was sequenced with a combination of T7 sequencing primers, the M13 (−40) primer, and chemically synthesized 17-mer oligonucleotide primers. After sequence analysis of both strands (see Materials and Methods; Table 2), the only mutation detected was a guanine-to-adenine transition at position 397 (35). The transition mutation results in the substitution of a basic lysine residue for an acidic glutamate residue at amino acid 125. The insertion of an amino acid with a basic side chain could potentially disrupt a salt bridge. To verify that this G-to-A transition was responsible for the mutation, a *trp-lac* [P_{tac}-*ftsQ1*(Ts)] transcriptional fusion was constructed in plasmid pKK223-3. This construction (pDSC77) is identical to a previously described plasmid (pDSC78, *ftsQ*$^+$ [39]), except it contains the *ftsQ1*(Ts) allele. Plasmid pDSC77 does not complement a chromosomal *ftsQ1*(Ts) strain with or without induction of the *ftsQ1*(Ts) plasmid allele (we discuss this experiment in another context later in this paper). We then replaced the 620-bp *Kpn*I fragment of plasmid pDSC77 with the same *Kpn*I fragment from the wild-type *ftsQ* of plasmid pDSC78. The wild-type fragment restored the complementation activity. Furthermore, the DNA sequence of this region of the wild-type *ftsQ* gene, on plasmid pDSC73 (39), was determined to verify that our *ftsQ*$^+$ gene does not contain the mutation. Figure 2 is an autoradiograph from a sequencing gel comparing the nucleotide sequences of the regions from the nucleotides 369 to 423 (35) in the wild-type and mutant alleles.

Isolation and mapping of extragenic suppressors of *ftsQ1*(Ts): a summary. We isolated extragenic suppressors of strain AMC436 *ftsQ1*(Ts) (see Materials and Methods) and then attempted to map the temperature-resistant phenotype by transposing a defective Tn*10* (tetracycline resistance) into the chromosome at random and mapping those Tn*10* insertions that were linked to temperature resistance. Surprisingly, one Tn*10*-containing derivative of strain AMC436-64 yielded 100% cotransduction between tetracycline resistance and temperature resistance when strain AMC436 *ftsQ1*(Ts) was the recipient. One of these Tn*10*-containing transductants of AMC436 *ftsQ1*(Ts) was designated strain AMC501. Further mapping demonstrated that this Tn*10* was not linked to the 2-min region where the *ftsQ1*(Ts) maps. In fact, Tn*10* of strain AMC501 alone produced suppression of *ftsQ1*(Ts). The possibility that the original weak suppressor mutation (designated *suqA* in strain AMC436-64) was in the region where Tn*10* of strain AMC501 maps was later ruled out when Tn*10* was precisely mapped (Tables 3 and 4). We have never precisely mapped *suqA*. We have attempted to minimize the confusion between *suqA* and Tn*10* of strain AMC501 by removing the data on *suqA* when possible. We now present details of the experiments concerning the isolation and mapping of the Tn*10* suppressor in strain AMC501, the major subject of this paper.

TABLE 3. P1-mediated mapping of Tn*10*

Donor strain	Recipient strain	Unselected marker[a]	% Cotransduction (no. unselected/ no. selected)
AMC501	E177	Temperature[b] (*dnaA*$^+$)	20 (8/40)
AMC501	RE74	*metB*$^+$	0 (0/78)
		pyrE$^+$	0 (0/78)
AMC501	AB2545	*metE*$^+$	8 (6/77)
AMC501	JF448	*asnA*$^+$	90 (146/156)
		bglR^{+c}	13 (10/76)
AMC501	JP58	*ilvC*$^+$	98 (152/156)
		argE$^+$	0 (0/78)

[a] The selected marker for all strains was Tn*10*.
[b] Ability to grow on LB at 42°C.
[c] Scored by inability to grow on M9 containing asparagine and salicin.

Isolation and chromosomal mapping of Tn*10* in strains AMC501, AMC502, and AMC503. A defective Tn*10* was randomly transposed into the chromosome by infecting a culture of AMC436-64 with λ1098. Approximately 6,000 tetracycline-resistant clones were obtained after overnight incubation at 42°C on LB-tetracycline plates. P1 *vir* was grown on a suspension of the 6,000 tetracycline-resistant colonies. The resulting P1 lysate was used to transduce AMC436 *ftsQ1*(Ts) to tetracycline resistance. These tetracycline-resistant transductants were then scored for temperature resistance by streaking on TEY at 41°C. Three temper-

TABLE 4. Cotransduction of *ilv*$^+$ and suppression of *ftsQ1*(Ts)

Donor strain	Recipient strain	Unselected marker[a]	% Cotransduction (no. unselected/ no. selected)
AMC501 [*rho* promoter::Tn*10* *ftsQ1*(Ts)]	DSC23 (*ftsQ1*(Ts) *ilvY*::Tn*10*)	Temp^{rb}	78 (81/104)
AMC436-64 [*ftsQ1*(Ts) *suqA*]	DSC23	Temp^r	0 (0/104)
Morse 2055 (*rho-4*)	DSC23	Temp^r	74 (77/104)

[a] The selected marker for all strains was *ilvY*$^+$.
[b] Ability to grow on LB medium without NaCl at 40°C on streak plates.

ature-resistant, tetracycline-resistant transductants were identified. P1 *vir* was grown on the three purified clones and used to transduce AMC436 *ftsQ1*(Ts) to tetracycline resistance. The resulting transductants were then scored for temperature resistance. One of the P1 lysates cotransduced tetracycline resistance and temperature resistance at a frequency of 100%. A temperature-resistant, tetracycline-resistant clone obtained from the transduction was designated AMC501. Our initial hypothesis was that *suqA* resulted from inactivation of gene function at an undefined locus and that Tn*10* transposed into this nonfunctional locus. An alternative hypothesis, that later proved to be correct, was that Tn*10* created a new suppressor locus. The other two lysates cotransduced the markers with a frequency of approximately 60%. Temperature-resistant, tetracycline-resistant transductants obtained from these two lysates were designated AMC502 and AMC503. To determine whether temperature resistance was due to reversion at the *ftsQ* locus, P1 grown on AMC501, AMC502, and AMC503 was used to transduce strain PK3 *leuB6* to tetracycline resistance. The transductants were then scored for leucine prototrophy. P1(AMC501) demonstrated no linkage between the Tn*10* and *leuB*. The P1(AMC502) and P1(AMC503) lysates cotransduced the Tn*10* and *leuB* alleles at frequencies of 97 and 64%, respectively. Because Tn*10* mapped in the 2-min region of the chromosome in these two strains, we assume a subpopulation of either the AMC436 or the AMC436-64 culture reverted at the *ftsQ* locus during the isolation of AMC502 and AMC503; thus, AMC502 and AMC503 were not characterized further.

A problem encountered during the isolation of extragenic suppressors is the potential for acquiring suppressor tRNAs. The *ftsM1* mutation was recently shown to be allelic with $tRNA_2^{Ser}$ (21), and *div* E42 was identified as a $tRNA_1^{Ser}$ suppressor (41). To evaluate our strains, the titers of several amber, ochre, and opal bacteriophages were determined on AMC436, AMC436-64, and AMC501. None of the phages were capable of growing on these strains (data not shown).

To map the Tn*10* insertion in strain AMC501, a time-of-entry experiment was performed by using PK3 Hfr::Tn*10* (strain PK3 Hfr transduced to tetracycline resistance with strain AMC501; see Material and Methods) as the donor and AMC426 as the recipient in a conjugation experiment. The time of entry was approximately 7 min, tentatively placing Tn*10* at 84 min on the *E. coli* chromosome.

Mapping of Tn*10* of strain AMC501 by bacteriophage P1 transduction. A number of strains with genetic markers near the 84-min region of the *E. coli* chromosome were transduced to tetracycline resistance with P1 grown on strain AMC501. The tetracycline-resistant transductants were then scored to determine the percent linkage of the relevant genetic markers (Table 3). Tn*10* was 98% linked to the *ilv* C7 allele in strain JP58. Because the transduced strain had no isoleucine or valine auxotrophy and because the *ilv* operon is fairly large (~4 kb), we postulated that Tn*10* was immediately clockwise to the *ilvC* gene (Fig. 3). This region of the *E. coli* chromosome has been extensively characterized. Immediately clockwise to *ilvC* are the *rep*, *trxA*, and *rho* genes (5, 37, 42). The *rep* gene product, a helicase, is essential for the replication of filamentous phages such as M13mp19 (reviewed in reference 40), and strain JM101 is a host for M13mp19. Strain JM101 was transduced to tetracycline resistance with P1 grown on strain AMC501. Thereafter, the titers of an M13mp19 lysate were determined on JM101 and on the two tetracycline-resistant isolates obtained by transducing JM101 to tetracycline resistance with P1(AMC501).

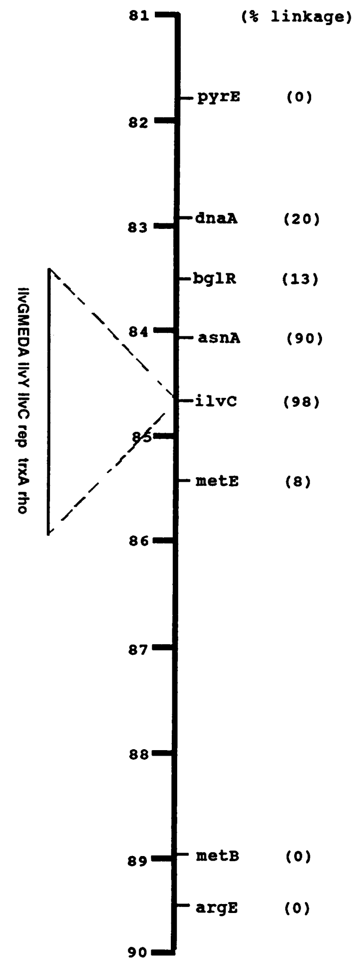

FIG. 3. The 84-min region of the *E. coli* chromosome (3). The cotransduction frequency between Tn*10* and the genetic markers is indicated in parentheses.

The titer of an M13mp19 lysate was $4.5 \times 10^{11} \pm 0.7 \times 10^{11}$ PFU per ml on both strain JM101 and the JM101 Tn*10*-containing strains, suggesting that Tn*10* was not inserted within the *rep* gene. The *trxA* gene encodes thioredoxin, an essential subunit for the T7 DNA polymerase (27). The *trxA* gene product is not essential for *E. coli* growth but is required for phage T7 replication (18). The ability of AMC436, AMC436-64, and AMC501 to support the growth of T7 was evaluated. The T7 titer was $4.0 \times 10^9 \pm 0.6 \times 10^9$ PFU per ml of lysate for all three strains tested. Thus, we concluded Tn*10* had not disrupted *trxA*.

Mapping Tn*10* of strain AMC501 by DNA sequencing. Because of our inability to precisely map Tn*10* by using P1 transduction techniques, an alternative approach was adopted. A bacteriophage (λDSC100) was isolated that contained Tn*10* and adjacent chromosomal DNA sequences from AMC501. After λDSC100 DNA was subcloned into M13, clones of M13 containing AMC501 DNA that hybridized with oligonucleotides specific for IS*10R* were examined by DNA sequence analysis (see Materials and Methods). By using the M13 (−40) and IS*10R*-specific oligonucleotides as sequencing primers (Table 2), the Tn*10* insertion was localized to the −35 consensus sequence of the *rho* gene, with Tn*10* oriented such that transcription from the *tetR* gene is towards *rho* (Fig. 4). Insertion of Tn*10* does not regenerate a

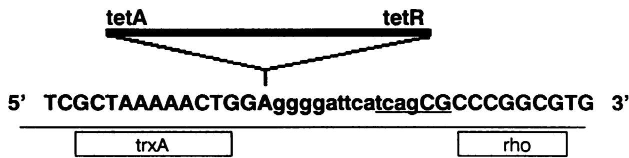

FIG. 4. DNA nucleotide sequence of the Tn*10* insertion point in AMC501. The uppercase letters denote the nucleotide sequence of the *E. coli* genome (22, 33, 36, 42). The lowercase letters represent the sequence of the right side of Tn*10* (13). The underlined sequence is the region where a new −35 sequence could have been formed but apparently was not. The original −35 of *rho* is CTGGACG, the five capital letters to the left and two capital letters to the right of the indicated tetA-tetR (Tn*10*) insertion.

consensus −35 sequence (Fig. 4, underlined region). Because *rho* appears to be an essential gene in *E. coli* (7, 20), this was a surprising finding. Therefore, it is probable that readthrough transcription from the *tetR* gene is sufficient for Rho protein synthesis. The *trxA* transcript has been shown to overlap the −35 region of *rho* (23, 34, 37, 43). On the basis of the ability of AMC501 to support the growth of phage T7, insertion of Tn*10* into the 3' noncoding region of the *trxA* transcript does not have a detrimental effect upon thioredoxin synthesis.

Further transduction mapping. Because Tn*10* and suppression of *ftsQ1*(Ts) were 100% linked, we needed to discern whether the *rho*:promoter:Tn*10* insertion was responsible for suppression of the *ftsQ1*(Ts) mutation or whether it simply mapped near the original *suqA* locus. Strain DSC23 *ftsQ1*(Ts) *ilvY*::Tn*10* was transduced to ilv^+ with P1(AMC 436-64), P1(AMC501), and P1(Morse 2055 *rho-4*). The transductants were then scored for temperature sensitivity [and tetracycline resistance for the P1(AMC501) transduction]. The experimental results are summarized in Table 4. The observed linkage between suppression and isoleucine-valine prototrophy (74 to 78%) was consistent with published values for the linkage between *rho* and *ilvY* (19, 42). No linkage was observed between *suqA* and *ilvY*; therefore, we conclude that the *suqA* gene maps elsewhere on the *E. coli* chromosome. To quantify the level of suppression, plating efficiencies were determined for the rho^+ and *rho* mutant strains on LB and TEY at 40°C. As demonstrated in Table 5, the *rho-4* and *rho* promoter::Tn*10* alleles both efficiently suppressed the *ftsQ1*(Ts) mutation. However, the colonies were smaller than those observed with the wild-type strain AMC434. Strain DSC24 *rho-4* apparently suppressed better than AMC501, as indicated by the larger colony size after a 19-h incubation on TEY. In fact, the AMC501 colonies required an extended incubation (42 h) before they were large enough to facilitate a reliable plate count. The wild-type strain, AMC434, was transduced to tetracycline resistance with P1(AMC501) and tested for the ability to grow on LB and TEY at 40°C. There was no significant difference in the growth rate on the two media. Therefore, the slow growth rate of AMC501 on TEY at 40°C can be attributed to weak suppression, as opposed to any detrimental effects associated with insertion of Tn*10* into the *rho* gene. The data in Table 5 also demonstrate that the *ftsQ1*(Ts) *suqA* strain AMC436-64 cannot be distinguished from the *ftsQ1*(Ts) strain AMC436 when individual cells are plated. Nevertheless, the temperature resistance of AMC436-64 could be recognized on streak plates of the same medium (TEY) incubated at 40°C from the same inoculating culture. It was this finding that discouraged our further studies of the *suqA* allele.

TABLE 5. Suppression of the *ftsQ1*(Ts) temperature-sensitive phenotype

Strain	Genotype	Temp sensitivity[a]
AMC434	$ftsQ^+$	0.89
AMC436	*ftsQ1*(Ts)	1×10^{-4}
AMC436-64	*ftsQ1*(Ts) *suqA*	0.9×10^{-4}
AMC501	*ftsQ1*(Ts) *rho* promoter::Tn*10*	0.38
DSC23	*ftsQ1*(Ts)	1×10^{-4}
DSC24	*ftsQ1*(Ts) *rho-4*	0.53

[a] Ratio of CFU on LB without NaCl to CFU on LB at 40°C.

It is difficult to explain the suppression of the *ftsQ1*(Ts) mutation by either *rho-4* or *rho* promoter::Tn*10*. *rho* mutations are defined by their ability to suppress polar nonsense mutations (reviewed in reference 46). This suppression is attributed to inefficient transcription termination at Rho-dependent termination sites (46). The *ftsQ1*(Ts) mutation is not expected to fall into the category of Rho-suppressible mutations. Because *ftsQ1*(Ts) mutant strains display a salt-remediable, temperature-sensitive phenotype, it was assumed that they contain a missense as opposed to a nonsense mutation. Our sequencing of the *ftsQ1*(Ts) and the wild-type alleles (Fig. 2) confirmed these expectations. Furthermore, a plasmid-encoded wild-type *ftsQ* gene complements the *ftsQ* mutation in an *ftsQ1*(Ts) *recA56* mutant strain regardless of whether it is induced by isopropyl-β-D-galactopyranoside (IPTG) (39). These data are consistent with the hypothesis that the only detrimental effect of the temperature-sensitive mutation is upon the *ftsQ* gene product, not the downstream *ftsA* or *ftsZ* genes.

Is there a *rho* mutant phenotype associated with *rho* promoter::Tn*10*? In an effort to determine the effect of the Tn*10* insertion into the −35 region of the *rho* gene, the phenotype of AMC501 was evaluated. We examined the ability of our *rho* promoter::Tn*10* mutation to suppress polar mutations both in the galactose operon (7) and in the tryptophan operon (31). Strain IT1022 *galP3 ilv*::Tn*10 his* was transduced to ilv^+ with P1 grown on AMC434, AMC436, AMC436-64, and AMC501. Transfer of the *rho* promoter::Tn*10* allele in the transductants derived from AMC501 was verified by testing for tetracycline resistance. Seventy-eight ilv^+ colonies from each transduction were then tested for polar suppression of the *galP3* mutation on the basis of the ability of suppressed strains to form red colonies on MacConkey's galactose (1%) medium because of reduced transcription termination. None of the transductants utilized galactose as a carbon source. As a control, when the *rho-4* allele in Morse 2055 was transduced into strain IT1022, red galactose-positive colonies were obtained at high frequency. Suppression of the polar *trpE9851* ochre mutation permits growth on medium containing anthranilate (31, 32). Therefore, P1(AMC501) was used to transduce Morse 2034 *trpE9851 leu-277*. Under conditions in which 160 tetracycline-resistant transductants were obtained per plate, no colonies were obtained on M9 medium supplemented with leucine and anthranilate. Thus, the *rho*::Tn*10* allele did not suppress polarity in either the *gal* or *trp* operon.

An effect of *rho* promoter::Tn*10* and *rho-4* on bacteriophage λN-independent growth. It has been demonstrated that some *rho* mutants inefficiently support the growth of λN mutants (11). Therefore, we determined the titers of several λN mutants on AMC434, AMC436, AMC436-64, AMC501,

TABLE 6. Effect of the *rho* promoter::Tn*10* and *rho-4* mutations on λ plaquing efficiency

Bacterial host	Genotype	Titer in host of λ phage genotype:			
		λ^+	λN7	λN7N53	λN7N53*nin5*
NK5012	*supE rho*$^+$	1.00	1.00	1.00	1.00
AMC434	*sup*^{0} *rho*$^+$	0.95	6×10^{-7}	0	$\sim 5 \times 10^{-4}$
AMC436	*sup*^{0} *rho*$^+$	0.95	4×10^{-7}	0	$\sim 5 \times 10^{-4}$
AMC436-64	*sup*^{0} *rho*$^+$	1.05	7×10^{-7}	0	2×10^{-3}
AMC501	*sup*^{0} *rho* promoter::Tn*10*	0.81	5×10^{-7}	0	0.94
DSC23	*sup*^{0} *rho*$^+$	0.71	4×10^{-7}	0	$\sim 5 \times 10^{-4}$
DSC24	*sup*^{0} *rho-4*	0.97	7×10^{-7}	0	0.83

DSC23, and DSC24. The results are presented in Table 6. All of the phages grew well on the *supE* mutant strain NK5012, as expected. However, none of the experimental bacterial strains propagated either λN7 or λN7N53 well enough to produce plaques, including *rho* promoter::Tn*10* and *rho-4*. These results are not particularly surprising, since not all *rho* mutants are expected to propagate λN mutants (11). The λ*nin5* mutation deletes approximately 5% of the λ chromosome between the P and Q genes, including the terminator sequence t_{R2} and allows limited growth of λN mutants (11). Bacteriophage λN7N53*nin5* produces plaques with an efficiency of approximately 5×10^{-4} on our *rho*$^+$ strains. In contrast, the same λ produces plaques on both the *rho* promoter::Tn*10* and the *rho-4* strains with an efficiency approaching 1 (Table 6). This is the only phenotype in which these two *rho* alleles behave similarly. We will present interpretations of these findings below.

Mutations in *rho* are frequently associated with hypersensitivity to rifampin (13). Therefore, we tested the sensitivity of AMC434, AMC436, AMC436-64, and AMC501 to rifampin. All of the strains were sensitive to as little as 5 μg of rifampicin per ml; thus, no conclusions could be drawn from the experiments.

Plasmid supercoiling. Recent evidence demonstrates that *rho* mutations can have a profound effect upon the level of supercoiling in cells (1, 10). Because no demonstrable *rho* phenotype was found associated with the insertion of Tn*10* in the −35 region of *rho*, the degree of plasmid supercoiling in AMC501 was examined. Plasmid DNA isolated from AMC434, AMC436, AMC436-64, AMC501, and DSC25 was subjected to chloroquine gel electrophoresis. The *rho* promoter::Tn*10* mutation in AMC501 and DSC25 had a dramatic effect upon the degree of plasmid supercoiling (Fig. 5). Plasmid DNA isolated from these strains was substantially more negatively supercoiled than DNA isolated from AMC434, AMC436, or AMC436-64, as reflected by an increase of 4 in the linking number. These data are in contrast to data normally found with *rho* mutants. It had been demonstrated that *rho* mutants frequently exhibit decreased negative supercoiling (1, 10). We were not able to recover plasmid pBR322 from a *rho-4* strain in several attempts, thus preventing our direct examination of the supercoiling of plasmid in this strain.

A remaining question which we do not answer in this paper but which is of considerable interest is the mechanism by which *rho* promoter::Tn*10* causes increased negative supercoiling. The *rho* gene autoregulates its own transcription within narrow limits (6, 28). The *rho* promoter::Tn*10* insertion does not create a recognizable new −35 consensus sequence at the site of insertion (Fig. 4, underlined sequence). The insertion might reduce autoregulation and lead to increased synthesis of *rho* mRNA if transcription started either within the *tetR* gene or at another upstream site. Either the resulting protein might be more active (a Rho fusion protein) or there might be more of the wild-type Rho protein. If the Rho protein activity in the cell were higher than normal, increased negative supercoiling might result.

Models and their testing. One model to explain why either increased (*rho* promoter::Tn*10*) or presumably decreased negative supercoiling (*rho-4*) could suppress the *ftsQ1*(Ts) allele is that local altered chromosomal supercoiling in the vicinity of *ftsQ1*(Ts) might increase transcription and therefore translation of the mutant allele; a similar, more traditional model would be that perhaps there is a (second) polar nonsense mutation in the *ddl* gene immediately upstream of the *ftsQ1*(Ts) mutation and that mutations in *rho* would suppress the temperature-sensitive phenotype of the *ftsQ1*(Ts) allele by causing polarity suppression (36). Again, increased transcription and translation of the FtsQ1(Ts) protein would result. Both models were tested by varying the amplification of the transcription and translation of *ftsQ1*(Ts) as follows. A *trp-lac* (P_{tac})-*ftsQ1*(Ts) transcrip-

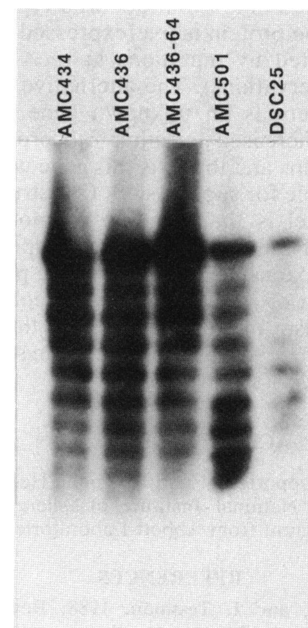

FIG. 5. The degree of plasmid pBR322 supercoiling in *rho*$^+$ (AMC434, AMC436, and AMC436-64) and *rho* promoter::Tn*10* (AMC501 and DSC25) strains. Plasmid DNA was isolated from the strains, blotted onto GeneScreen, and hybridized with radiolabeled pBR322 DNA.

tional fusion was constructed in plasmid pKK223-3, designated pDSC77. By either model described above, plasmid pDSC77 should suppress the temperature-sensitive phenotype of the *ftsQ1*(Ts) strain under some conditions in which the expression of the plasmid-derived copy of the $FtsQ^{ts}$ protein in *trans* is varied. Overexpression of the *ftsQ1*(Ts) allele from the P_{tac} promoter by induction with IPTG at maximal, intermediate, and zero levels did not restore growth at 40°C on complex medium without added salt (TEY medium). Growth on media with salt was not inhibited by IPTG at 40°C. Previously, we demonstrated that overexpression of the *ftsQ* wild-type allele had no deleterious effect when either complementing an *ftsQ1*(Ts) allele or in an *ftsQ* wild-type strain on TEY media at any temperature with either no IPTG or optimal amounts for induction (39; unpublished results). We have directly demonstrated the regulated synthesis of the FtsQ protein when it was under control of the P_{tac} promoter (39). Thus, both models are no longer attractive.

In this paper we have reported one previously undescribed phenotype of the *rho-4* and *rho* promoter::Tn*10* alleles; they allow bacteriophage λN7N53*nin5* to plate with an efficiency approaching 1 (Table 6). While this finding can be interpreted as reducing polarity and causing increased transcription through remaining Rho protein-dependent terminators for *rho-4* at least (11, 38, 46), the molecular mechanism of this suppression may be dependent on the superhelical state of the λ and/or chromosomal DNA.

At present, our working model to explain suppression of *ftsQ1*(Ts) by the *rho* promoter::Tn*10* and *rho-4* alleles is that the FtsQ protein specified by the *ftsQ1*(Ts) allele interacts more readily with DNA of either increased (*rho* promoter::Tn*10*) or presumably decreased (*rho-4*) negative superhelicity and is thereby stabilized in its function in the inner membrane (39). The hypothetical interaction of the FtsQ protein with DNA is supported by our finding that a weak SOS response, including reduced colony size and filamentous growth, occurs in *lon* (but not wild-type) strains when the FtsQ wild-type protein is overexpressed; the weak SOS response is negated by mutations in *recA*, *sulA*, or *sulB* (unpublished observations). The alternative to our working model is that there is an unknown gene (*suqA?*) whose transcription is increased in either *rho* promoter::Tn*10* or *rho-4* mutant strains and that it is this gene whose product is directly responsible for suppression. Our strongest evidence against this model is that our *rho* promoter::Tn*10* allele exhibited none of the suppressor phenotypes of *rho* mutations despite an extensive search for these phenotypes. The weak suppression of *ftsQ1*(Ts) by *suqA* could be due to a different mechanism of suppression than that of either *rho* promoter:Tn*10* or *rho-4*, since no change in supercoiling was detected in the *suqA* strain.

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