A Novel rho Promoter::Tn10 Mutation Suppresses an ftsQI(Ts) Missense Mutation in an Essential Escherichia coli Cell Division Gene by a Mechanism Not Involving Polarity Suppression

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An extragenic suppressor of the Escherichia coli cell division gene ftsQI(Ts) was isolated. The suppressor is a Tn10 insertion into the −35 promoter consensus sequence of the rho gene, designated rho promoter::Tn10. The ftsQI(Ts) mutation was also suppressed by the rho− mutant allele. The rho promoter::Tn10 strain does not exhibit rho mutant polarity suppressor phenotypes. In addition, overexpression of the ftsQI(Ts) mutation does not reverse temperature sensitivity. Furthermore, DNA sequence analysis of the ftsQI(Ts) allele revealed that the salt-remediable, temperature-sensitive phenotype arose from a single missense mutation. The most striking phenotype of the rho promoter::Tn10 mutant strain is an increase in the level of negative supercoiling. On the basis of these observations, we conclude that the ftsQI(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 (subB, sfbB), and ftsI (sep, phbB) 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 effect 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 ftsQI(Ts). In this report, we characterize the ftsQI(Ts) mutation and demonstrate that mutations in either the rho gene or its promoter are capable of suppressing the ftsQI(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 CaCl2 supplemented with amino acids (50 μg/ml) as necessary. LB medium lacking NaCl (TEY) was used to determine the temperature sensitivity of the ftsQI(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. Tn10 transposition into the chromosome from λ1098 was performed as recommended by Way et al. (45). This phage contains a defective Tn10 that is only capable of a single transposition event. For initial mapping of Tn10 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::Tn10 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 ftsQI(Ts). Strain AMC436 ftsQI(Ts) was plated on TEY at 42°C at 106 to 107 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 [mutations::Tn10::ftsQI(Ts)]. The leu−:Tn10 marker is 70% cotransduced with ftsQI(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-
### TABLE 1. Bacterial strains and phages

| Strain or phage | Relevant genotype and phenotype | Source (reference) |
|-----------------|---------------------------------|-------------------|
| **E. coli K-12** | recA1 Δ(lac-pro) thi supEF lacProAB ΔZDM15 | BRL* |
| DH5α |  | BRL (29) |
| JM101 |  | This laboratory (39) |
| Q59 |  | This laboratory (39) |
| N5012 |  | This laboratory (39) |
| SG13022 |  | This laboratory (39) |
| TOE-1 |  | This laboratory (39) |
| AB 259 |  | This laboratory (39) |
| MC169 |  | This laboratory (39) |
| AMC290 |  | This laboratory (39) |
| AMC405 |  | This laboratory (39) |
| AMC416 |  | This laboratory (39) |
| AMC424 |  | This laboratory (39) |
| AMC426 |  | This laboratory (39) |
| AMC434 |  | This laboratory (39) |
| AMC436 |  | This laboratory (39) |
| AMC436-64 |  | This laboratory (39) |
| AMC501 |  | This laboratory (39) |
| AMC502 |  | This laboratory (39) |
| AMC503 |  | This laboratory (39) |
| DSC23 |  | This laboratory (39) |
| DSC24 |  | This laboratory (39) |
| DSC25 |  | This laboratory (39) |
| IT1022 |  | This laboratory (39) |
| Morse 2034 |  | This laboratory (39) |
| Morse 2055 |  | This laboratory (39) |
| JP58 |  | This laboratory (39) |
| PK3 |  | This laboratory (39) |
| E177 |  | This laboratory (39) |
| RE74 |  | This laboratory (39) |
| JF448 |  | This laboratory (39) |
| AB2545 |  | This laboratory (39) |

* BRL, Bethesda Research Laboratories.

### Phage

| Phage | Relevant genotype and phenotype | Source (reference) |
|-------|---------------------------------|-------------------|
| P1 vir |  | Laboratory stock |
| M13mp19 |  | BRL |
| T7 |  | Laboratory stock |
| λ1098 |  | λEMBL3 |
| λEMBL3 |  | Stratagene (12) |
| λDSC100 |  | Contains the allele from AMC501 |
| λDSC104 |  | This study |
| λ c857 |  | D. Kaiser |
| λ c857 N7 |  | A. Campbell via W. Epstein |
| λ c857 N7 N53 |  | A. Campbell via W. Epstein |
| λ c857 N7 N53 nin5 |  | D. Court |

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The nature-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 *ftsQ*(Ts) allele and thus harbored a suppressor(s) of *ftsQ*(Ts) that failed to map to the 2-min region of the *E. coli* chromosome. One of these, strain AMC436-64 [designated *ftsQ*(Ts) *suqA*], was selected for further characterization.

**Isolation of recombinant λ phages.** Chromosomal DNA isolated from AMC436 and AMC501 was partially digested with *Sal*III and cloned into *Bam*HI-digested EMBL3 phage DNA as described by Frischau 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* Q539 to select for recombinant phage. Plaques onto nitrocellulose and hybridization conditions were as recommended by the supplier (Schleicher & Schuell, Inc.). The AMC436 library was screened with a 970-bp *EcoR*I-PvuII fragment, which was isolated from pDS73 (39) and labeled with α-32pDCTP 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-32pDCTP-labeled pNK82 (14). This hybridization probe contains *Tn10* sequences from the *IS10* to the *EcoR*I 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 pDS73 and pDS78 have been described previously (39). Plasmid pDS75 contains a 2,470-bp *Xho*I-HindIII fragment isolated from...
\(\lambda\)DSC104 cloned into Sall-HindIII-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 SmaI and PvuII and cloning the 1,640-bp fragment into HindIII-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 DH5\(\alpha\) (16). Large-scale isolation of plasmid DNA from DH5\(\alpha\) 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 \(\lambda\)DSC100 phage was partially digested with Sau3A, and the fragments were subcloned into M13mp19. The M13mp19 clones were screened with oligonucleotides complementary to the outside end of IS10R inverted repeats bracketing the Tn10 (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 IS10R.

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 \(\mu\)g 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 \([\alpha-\text{32P}]d\text{CTP}\) 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 \(Xho1\) 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' AACTGCCAGGAAAGCCCG 3' | 35 |
| \(ftsQ\) | 5' CGTTCTGGCGCAGCTG 3' | 35 |
| \(ftsQ\) | 5' AATTGGCGTCACCGGTC 3' | 35 |
| \(ddl\) | 5' TACCTGGGTCGCTG 3' | 35 |
| \(ddl\) | 5' GGGTCCGAAAGTTTGA 3' | 35 |
| IS10R | 5' CTGATGAATCCCTTAATGATT 3' | 14 |
| IS10R | 5' AAAATCATTAGGGATTCAG 3' | 14 |
to the PvuII 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 ddd 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 ExolIII-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 [Pac-ftsQl(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 ftsQl(Ts) allele. Plasmid pDSC77 does not complement a chromosomal ftsQl(Ts) strain with or without induction of the ftsQl(Ts) plasmid allele (we discuss this experiment in another context later in this paper). We then replaced the 620-bp KpnI fragment of plasmid pDSC77 with the same KpnI 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 ftsQl(Ts): a summary. We isolated extragenic suppressors of strain AMC436 ftsQl(Ts) (see Materials and Methods) and then attempted to map the temperature-resistant phenotype by transposing a defective Tn10 (tetracycline resistance) into the chromosome at random and mapping those Tn10 insertions that were linked to temperature resistance. Surprisingly, one Tn10-containing derivative of strain AMC436-64 yielded 100% cotransduction between tetracycline resistance and temperature resistance when strain AMC436 ftsQl(Ts) was the recipient. One of these Tn10-containing transductants of AMC436 ftsQl(Ts) was designated strain AMC501. Further mapping demonstrated that this Tn10 was not linked to the 2-min region where the ftsQl(Ts) maps. In fact, Tn10 of strain AMC501 alone produced suppression of ftsQl(Ts). The possibility that the original weak suppressor mutation (designated suqA in strain AMC436-64) was in the region where Tn10 of strain AMC501 maps was later ruled out when Tn10 was precisely mapped (Tables 3 and 4). We have never precisely mapped suqA. We have attempted to minimize the confusion between suqA and Tn10 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 Tn10 suppressor in strain AMC501, the major subject of this paper.

Isolation and chromosomal mapping of Tn10 in strains AMC501, AMC502, and AMC503. A defective Tn10 was randomly transposed into the chromosome by infecting a culture of AMC436-64 with λ1098. Approximately 6,000 tetracycline-resistant colonies 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 ftsQl(Ts) to tetracycline resistance. These tetracycline-resistant transductants were then scored for temperature resistance by streaking on TEY at 41°C. Three temper-

![FIG. 2. Comparison of the DNA sequences of ftsQ+ (pDSC73) and ftsQl(Ts) (pDSC76). The point mutation is indicated by an arrow.](image)

| Donor strain | Recipient strain | Unselected marker* | % Cotransduction (no. unselected/ no. selected) |
|--------------|-----------------|-------------------|-----------------------------------------------|
| AMC501       | E177            | Temperaturea (dnaA+) | 20 (8/40)                                   |
| AMC501       | RE74            | metB+             | 0 (0/78)                                     |
| AMC501       | AB2545          | metE+             | 0 (0/78)                                     |
| AMC501       | JF448           | argE+             | 8 (6/77)                                     |
| AMC501       | JF448           | bgIR+             | 90 (146/156)                                 |
| AMC501       | JP58            | ilvC+             | 13 (10/76)                                   |
| AMC501       | JP58            | argE+             | 98 (152/156)                                 |
| AMC501       | JP58            | ilv+              | 0 (0/78)                                     |

* The selected marker for all strains was Tn10.

** Ability to grow on LB at 42°C.

+a Scoared by inability to grow on M9 containing asparagine and salicin.

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**TABLE 4. Cotransduction of ilv+ and suppression of ftsQl(Ts)**

| Donor strain | Recipient strain | Unselected marker* | % Cotransduction (no. unselected/ no. selected) |
|--------------|-----------------|-------------------|-----------------------------------------------|
| AMC501 [rho promoter::TnlO ftsQl(Ts)] | DSC23 (ftsQl(Ts)) | ilvY::Tn10 | 78 (81/104)                                 |
| AMC436-64 [ftsQl(Ts) suqA] | DSC23 | Temp | 0 (0/104)                                   |
| Morse 2055 [rho-4] | DSC23 | Temp | 74 (77/104)                                 |

* The selected marker for all strains was ilvY+.

** Ability to grow on LB medium without NaCl at 40°C on streak plates.
urate-resistant, tetracycline-resistant transductants were identified. P1 vir was grown on the three purified clones and used to transduce AMC436 fisQ1(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 sugA resulted from inactivation of gene function at an undefined locus and that Tn1O transposed into this nonfunctional locus. An alternative hypothesis, that later proved to be correct, was that Tn1O 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 fisQ 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 Tn1O and leuB. The P1(AMC502) and P1(AMC503) lysates cotransduced the Tn1O and leuB alleles at frequencies of 97 and 64%, respectively. Because Tn1O 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 fisQ 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 fisM1 mutation was recently shown to be allelic with tRNA^Ser^ (21), and div E42 was identified as a tRNA^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 Tn1O insertion in strain AMC501, a time-of-entry experiment was performed by using PK3 Hfr::Tn1O (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 Tn1O at 84 min on the E. coli chromosome.

**Mapping of Tn1O 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). Tn1O 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 Tn1O 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).

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**FIG. 3.** The 84-min region of the E. coli chromosome (3). The cotransduction frequency between Tn1O 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 Tn1O-containing strains, suggesting that Tn1O 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 Tn1O had not disrupted trxA.

**Mapping Tn1O of strain AMC501 by DNA sequencing.** Because of our inability to precisely map Tn1O by using P1 transduction techniques, an alternative approach was adopted. A bacteriophage (AdSC100) was isolated that contained Tn1O and adjacent chromosomal DNA sequences from AMC501. After AdSC100 DNA was subcloned into M13, clones of M13 containing AMC501 DNA that hybridized with oligonucleotides specific for IS10R were examined by DNA sequence analysis (see Materials and Methods). By using the M13 (~40) and IS10R-specific oligonucleotides as sequencing primers (Table 2), the Tn1O insertion was localized to the ~35 consensus sequence of the rho gene, with Tn1O oriented such that transcription from the tetR gene is towards rho (Fig. 4). Insertion of Tn1O does not regenerate a
FIG. 4. DNA nucleotide sequence of the Tn10 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 Tn10 (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 CTGGAGCG, the five capital letters to the left and two capital letters to the right of the indicated tetA-tetR (Tn10) 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 phase T7, insertion of Tn10 into the 3′ noncoding region of the trxA transcript does not have a detrimental effect upon thio- doxin synthesis.

Further transduction mapping. Because Tn10 and suppression of ftsQ1(Ts) were 100% linked, we needed to discern whether the rho promoter::Tn10 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::Tn10 was transduced to ilv + with P1(AMC436-64), 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 ilv Y (19, 42). No linkage was observed between suqA and ilv Y; 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::Tn10 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 Tn10 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.

It is difficult to explain the suppression of the ftsQ1(Ts) mutation by either rho-4 or rho promoter::Tn10. 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) recA36 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::Tn10? In an effort to determine the effect of the Tn10 insertion into the −35 region of the rho gene, the phenotype of AMC501 was evaluated. We examined the ability of our rho promoter::Tn10 mutation to suppress polar mutations both in the galactose operon (7) and in the antibiotic operon (31). Strain IT1022 galP3 ilv::Tn10 his was transduced to ilv + with P1 grown on AMC434, AMC436, AMC436-64, and AMC501. Transfer of the rho promoter::Tn10 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 antranilate (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 antranilate. Thus, the rho::Tn10 allele did not suppress polarity in either the gal or trp operon.

An effect of rho promoter::Tn10 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,

| Strain     | Genotype | Temp. sensitivity* |
|------------|----------|-------------------|
| AMC434     | ftsQ    | 0.89              |
| AMC436     | ftsQ1(Ts) | 1 x 10^{-4}     |
| AMC436-64  | ftsQ1(Ts) suqA | 0.9 x 10^{-4}  |
| AMC501     | ftsQ1(Ts) rho promoter::Tn10 | 0.38      |
| DSC23      | ftsQ(Ts) | 1 x 10^{-4}     |
| DSC24      | ftsQ1(Ts) rho-4 | 0.53         |

* Ratio of CFU on LB without NaCl to CFU on LB at 40°C.
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 AN7 or AN7N53 well enough to produce plaques, including rho promoter::Tn10 and rho-4. These results are not particularly surprising, since not all rho mutants are expected to propagate AN mutants (11). The XN5 mutation deletes approximately 5% of the λ chromosome between the P and Q genes, including the terminator sequence tr2 and allows limited growth of AN mutants (11). Bacteriophage AN7N53nin5 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::Tn10 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 at 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 Tn10 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::Tn10 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::Tn10 causes increased negative supercoiling. The rho gene autoregulates its own transcription within narrow limits (6, 28). The rho promoter::Tn10 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::Tn10) or presumably decreased negative supercoiling (rho-4) could suppress the fitsQl(Ts) allele is that local altered chromosomal supercoiling in the vicinity of fitsQl(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 fitsQl(Ts) mutation and that mutations in rho would suppress the temperature-sensitive phenotype of the fitsQl(Ts) allele by causing polarity suppression (36). Again, increased transcription and translation of the FitsQl(Ts) protein would result. Both models were tested by varying the amplification of the transcription and translation of fitsQl(Ts) as follows. A trp-lac (PStu)-fitsQl(Ts) transcrip-

### Table 6. Effect of the rho promoter::Tn10 and rho-4 mutations on λ plaquing efficiency

| Bacterial host | Genotype         | Titer in host of λ phage genotype: |
|---------------|-----------------|-----------------------------------|
|               |                 | λ*      | AN7  | AN7N53 | λN7N53 nin5 |
| NK5012        | supE rho+       | 1.00    | 1.00 | 1.00   | 1.00        |
| AMC434        | sup° rho+       | 0.95    | 6 × 10^-7 | 0     | ~5 × 10^-4   |
| AMC436        | sup° rho+       | 0.95    | 4 × 10^-7 | 0     | ~5 × 10^-4   |
| AMC436-64     | sup° rho+       | 1.05    | 7 × 10^-7 | 0     | 2 × 10^-3    |
| AMC501        | sup° rho promoter::Tn10 | 0.81 | 5 × 10^-7 | 0     | 0.94        |
| DSC23         | sup° rho+       | 0.71    | 4 × 10^-7 | 0     | ~5 × 10^-4   |
| DSC24         | sup° rho-4      | 0.97    | 7 × 10^-7 | 0     | 0.83        |

![FIG. 5. The degree of plasmid pBR322 supercoiling in rho+ (AMC434, AMC436, and AMC436-64) and rho promoter::Tn10 (AMC501 and DSC25) strains. Plasmid DNA was isolated from the strains, blotted onto GeneScreen, and hybridized with radiolabeled pBR322 DNA.](image)
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 ftsQl(Ts) strain under some conditions in which the expression of the plasmid-derived copy of the FtsQ protein in trans is varied. Overexpression of the ftsQl(Ts) allele from the P_pac 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 ftsQl(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_pac 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::Tn10 alleles; they allow bacteriophage λN753n5 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 ftsQl(Ts) by the rho promoter::Tn10 and rho-4 alleles is that the FtsQ protein specified by the ftsQl(Ts) allele interacts more readily with DNA of either increased (rho promoter::Tn10) 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 lom (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 (supAQ?) whose transcription is increased in either rho promoter::Tn10 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::Tn10 allele exhibited none of the suppressor phenotypes of rho mutations despite an extensive search for these phenotypes. The weak suppression of ftsQl(Ts) by suAQ could be due to a different mechanism of suppression than that of either rho promoter::Tn10 or rho-4, since no change in supercoiling was detected in the suAQ strain.

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