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Identification of New Genes in a Cell Envelope-Cell Division Gene Cluster of *Escherichia coli*: Cell Division Gene *ftsQ*

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We report the identification, cloning, and mapping of a new cell division gene, *ftsQ*. This gene formed part of a cluster of three division genes (in the order *ftsQ ftsA ftsZ*) which itself formed part of a larger cluster of at least 10 genes, all of which were involved in some step in cell division, cell envelope synthesis, or both. The *ftsQAZ* group was transcribed from at least two independent promoters.

In *Escherichia coli* there is a large cluster of genes, all of which are involved in some aspect of cell envelope synthesis, cell division, or both (5–8, 11, 12; H. J. W. Wijsman, Genetics **74**: S296, 1973). So far, eight different genes have been placed unambiguously within this region by the use of specialized transducing phages (5–7), and a number of other mutations of similar phenotype have been located less precisely within or in the neighborhood of this cluster (see reference 1). The purpose of this and the accompanying note (9) is to report the discovery and positions within the cluster of two new genes which, like those described hitherto, appear to be concerned specifically with cell envelope growth, septum formation, or both.

Previous studies on the *ftsA* gene have shown that it is located within this cluster (6, 10, 12), that the action of the *ftsA* protein (a polypeptide of molecular weight 50,000 [6]) is required only during septum formation (11), and that the synthesis of this protein appears to be required only during a short period immediately before the onset of septum formation (4). If *ftsA* protein is not made at the correct time in one cell cycle, then the formation of a septum is blocked until after the synthesis of the protein at the equivalent time in the next cycle (4). A search was therefore made for division genes which had a single required time of action in each cycle. To do this, an asynchronous population of UV-irradiated cells was grown at 30°C and shifted to 42°C for periods of 5 min at each successive mass doubling of the culture. After three such cycles, the cells were passed through a membrane filter (pore size, 14 μm) to enrich for abnormally long cells. Those few cells which were trapped by the filter were subjected to a further three cycles of growth at 30°C with 5-min pulses at 42°C as before and then filtered once more. The cells which were trapped on the filter were then plated on nutrient agar (Oxoid nutrient broth plus 50 μg of thymine per ml) at 30°C, and the resultant clones were screened for temperature-sensitive mutants by replica plating at 30 and 42°C. A number of such mutants (designated "TOE" mutants for "temperature-oscillation enrichment") were obtained.

TOE-1 obtained in this way formed colonies on Oxoid nutrient agar at 30°C but not at 42°C. This temperature sensitivity was abolished by 1% sodium chloride (as is that of several of the already known *fts* mutants [8]). TOE-1 grew and divided in liquid medium at 30°C at a rate similar to that of its parent (*E. coli* K-12 AB2497), although the cells were somewhat larger on the average than those of the parent strain. When an asynchronous population of TOE-1 was shifted from 30 to 42°C, all cell division stopped immediately (Fig. 1), but exponential cell growth continued for a minimum of three mass doublings so that long, filamentous cells were produced (Fig. 2). These filaments were multinucleate (data not shown), indicating that DNA replication and segregation were not affected at the temperature which was restrictive for division. The phenotype of TOE-1 is therefore that of an *fts* mutant in that it appears to be affected specifically in septum formation (8, 10).

The mutation responsible for the TOE-1 phenotype was found by P1 transduction to be 75% cotransducible with *leu* at 1.6 min on the genetic map of *E. coli* (1). This made it likely that the mutation was within the known cluster of division-related genes located at about 2 min (1, 5, 12, 13; H. J. W. Wijsman, Genetics **74**:S296, 1973). This was confirmed, and the locus was determined precisely by the use of specialized transducing phages for this region (6, 7; G. Hatfull and J. F. Lutkenhaus, unpublished data). These plaque-forming, integration-proficient λ phages were constructed in vitro by cloning restriction endonuclease fragments of the chromosome and extending or deleting these in vivo or in vitro so as to contain various fragments of

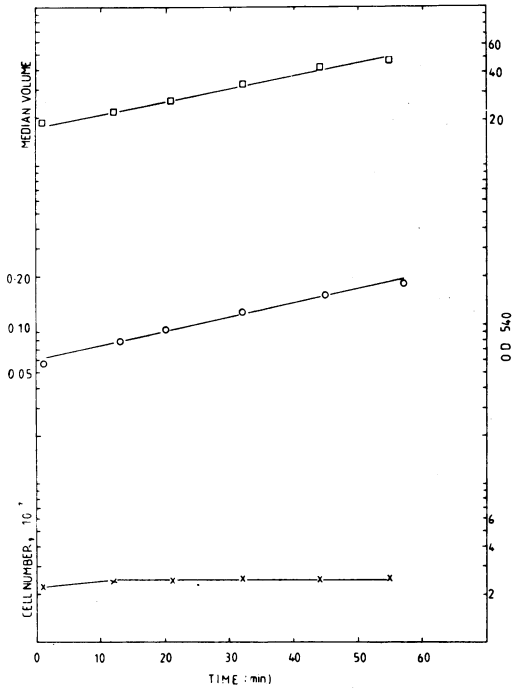

FIG. 1. *Kinetics of cell growth and division in a log-phase population of strain TOE-1 [ftsQ(Ts)] after a shift from 30 to 42°C at 0 min. Increase in total cell mass was followed as optical density (OD) at 540 nm* (○). *Cell number was measured with a Coulter electronic particle counter* (×), *and median cell volume* (□) *was measured with a Coulter Channelyzer, as described previously (2). Cell division stopped almost immediately after the shift, but cell growth continued.*

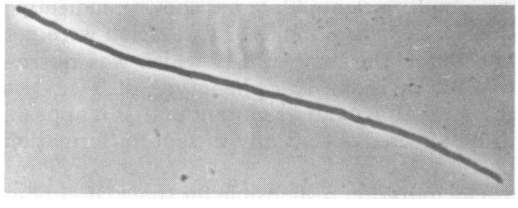

FIG. 2. *A cell of strain TOE-1 [ftsQ(Ts)] after four generations of growth at 42°C. The cell is approximately 64 μm in length, or about 10 to 20 times as long as cells grown at 30°C. Unlike the filamentous cells of ftsA(Ts) strains under comparable conditions, there is no sign of periodic indentations at presumptive septal sites (4, 6, 11).*

the region. These cloning vectors are especially convenient in that stable lysogens can be easily obtained (3). TOE-1 was lysogenized with a number of these at 30°C, and the lysogenic clones were tested for temperature sensitivity. The results obtained with two of the transducing phages (λ *ddl*$^+$ and λ FH16; Fig. 3) served to locate the TOE-1 mutation. Lysogenization with

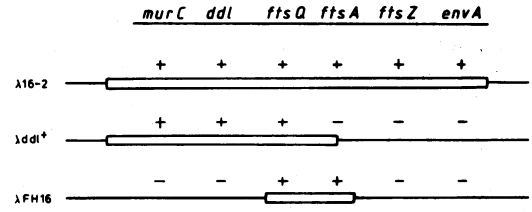

FIG. 3. *The order of genes in the murC-envA section of the E. coli chromosome (top) and the chromosomal inserts (boxes) cloned into the three specialized transducing phages, λ 16-2, λ ddl$^+$, and λ FH16. The "+" signs indicate that the gene directly above is complemented by the particular phage. The lengths of the inserts in the phages are approximately (from top to bottom) 9.77 × 10^3, 6.51 × 10^3, and 2.1 × 10^3 nucleotide base pairs. The region contains a minimum of three independent promoter sites.*

either of these two phages rendered TOE-1 temperature insensitive. The phenotypes of other lysogens made with phages carrying neighboring or overlapping regions were consistent with this location. When temperature-resistant lysogens were cured of their phages by the use of λ b2 (6) the cured cells were once again temperature sensitive. The right-hand end (as written) of the chromosomal insert in λ *ddl*$^+$ is defined by a *Hin*dIII site within the *ftsA* gene (6, 7) so that mutations within *ftsA* are not complemented by this phage. The chromosomal fragment cloned in λ FH16 was bounded by two *Eco*RI restriction sites (Fig. 3) and was already known to carry the entire *ftsA* gene (G. Hatfull, unpublished). The mutation in TOE-1 must therefore lie within this fragment in a new gene to the left of *ftsA*. Confirmation that this new mutation did not lie within the *ftsA* gene was obtained by lysogenizing TOE-1 with the phage λ 16-4, which carries the *ftsA12*(Ts) mutation on a larger fragment which covers the entire region in question (Fig. 3). Lysogenization of TOE-1 with λ 16-4 conferred a temperature-resistant phenotype. It could be argued that TOE-1 carries a missense mutation in *ftsA* and that λ *ddl*$^+$ produces a peptide fragment that confers temperature resistance by intragenic complementation. However, as λ *ddl*$^+$ did not complement any of the five *ftsA* alleles that we have tested, this is thought to be an unlikely possibility. Thus, we may conclude that TOE-1 carries a missense mutation in a new gene, which we designate *ftsQ*. The length of the chromosomal fragment cloned in λ FH16 is about 4.6% of lambda, or about 2.14 × 10^3 base pairs. The *ftsA* gene codes for a polypeptide of molecular weight 50,000 (6) which would require about 1.36 × 10^3 base pairs of DNA. The maximum length of the new gene (including its promoter) is about 700 to 800 base

pairs, giving a maximum molecular weight for the *ftsQ* product of about 20,000 to 30,000. It is not yet known whether *ftsA* shares this promoter or has one of its own. However, another independently expressed gene, *ftsZ*, has been located adjacent to *ftsA* on the other side (7) (Fig. 3), and this also is expressed on cloned fragments which do not carry a complete *ftsA* gene. Thus, *ftsA* may possibly share a common promoter with either *ftsQ* or *ftsZ*, but these latter two genes must have independent promoter sites. The known mutations in these three adjacent genes have very similar phenotypes in that they specifically affect late stages in septum formation. Nevertheless, it is clear that they form a minimum of two transcriptional units, each of which can be expressed efficiently when transposed away from its neighbors to an abnormal chromosomal location (att^{λ}). The functional reason, if any, for the close clustering of these cell division genes therefore remains unknown.

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