Effects of Chromosomal Inversion on Cell Fitness in *Escherichia coli* K-12

Charles W. Hill and Jane Antal Gray

Department of Biological Chemistry, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

Manuscript received November 20, 1987
Revised copy accepted April 15, 1988

ABSTRACT

In an effort to learn what factors might mitigate the establishment of *Escherichia coli* variants bearing major chromosomal rearrangements, we have examined the effects on cell growth of two inversions between rRNA operons. One of these inversions, IN(*rrnD-rrnE*), had been propagated in a commonly used subline of *E. coli* K-12 for approximately 30 yr before its discovery, a fact that illustrates the absence of obvious detrimental effects associated with the inversion. We found that culturing under conditions requiring repeated transition from stationary phase to rapid growth led to the replacement of IN(*rrnD-rrnE*) cells by cells that had undergone either of two types of additional chromosomal inversion: one type fully restored the wild-type order, while the other partially restored it. The partial reinversion was also between *rrn* operons, but it left a small transposition. The tendency for overgrowth by these revertants persisted through several rounds of periodic selection. In contrast, the other inversion, IN(*rrnG-rrnE*), was associated with severe, detrimental effects. The effects of IN(*rrnG-rrnE*) were also alleviated by full or partial reinversion. The probable relationship between the severity of the effects caused by the inversions and the degree of displacement of the replication origin is discussed. Spontaneous inversion events between *rrn* operons separated by 18% of the chromosome were estimated to occur at a frequency of roughly 10^{-5}. If extended to natural situations, the growth disadvantage together with the relatively high frequency of reinversion suggest that clones of cells with an inversion between these *rrn* operons would be readily overgrown by revertants.

THE preservation of the gross genetic organization of the enteric bacterial chromosome is a striking example of evolutionary conservation. The extent of this conservation has become apparent through comparison of the masses of data accumulated for both *Escherichia coli* and *Salmonella typhimurium*, showing the similarities of their maps (SANDERSON 1976; RILEY and ANILIONIS 1978). Contrasting with this stability of the chromosome through evolution is the high frequency of chromosomal rearrangement found in enteric bacterial cultures. The homology provided by gene families such as the ribosomal RNA operons (HILL *et al.* 1977; ANDERSON and ROTH 1978), insertion sequences (DAVIDSON *et al.* 1975; SAVIC, ROMAC and EHRLICH 1983; LOUARN *et al.* 1985; TIMMONS, LEIB and DEONIER 1986) and the *rhs* family (LIN, CAPAGE and HILL 1984) facilitates unequal recombination leading to duplications, deletions, transpositions, and inversions at substantial frequencies. Spontaneous duplications produced by recombination between *rrn* operons, for example, occur at frequencies in the range of 10^{-4} to 10^{-3} (HILL and COMBRIATO 1973; ANDERSON and ROTH 1978). Considering the high frequency of these major rearrangements, the stability of the chromosomal organization through evolution suggests that significant pressures exist to maintain the gross order (RILEY and ANILIONIS 1978; ROTH and SCHMID 1981). The major exception to this stability is the large chromosomal segment that includes the replication terminus which is inverted when the map of *S. typhimurium* is compared to that of *E. coli* (CASSE, PASCAL and CHIPPAUX 1973). In addition to this natural exception, large inversions have become established in laboratory strains, not to be discovered until years after their occurrence. For example, *E. coli* K-12 strain 1485IN acquired an inversion that covers 35% of the chromosome including the replication terminus (XIA and ENOMOTO 1986). An important subline of *E. coli* K-12, beginning with W2637 and including commonly used strains such as W3110 and W3102 (BACHMANN 1973), has acquired a large inversion that covers 18% of the chromosome and includes the replication origin (HILL and HARNISH 1981). This inversion, IN(*rrnD-rrnE*), was generated by recombination between the *rrnD* and *rrnE* operons which have opposite orientations within the chromosome (Figure 1). That this large inversion persisted for approximately 30 yr without detection in these widely used strains is testimony that any resulting detrimental effects are slight. The relative lack of harmful effects accompanying the IN(*rrnD-rrnE*) inversion would seem to be difficult to reconcile with the evolutionary stability of the chromosome. The objective of this work is to examine more closely the consequences to the cell of the IN(*rrnD-rrnE*) inversion as well as a larger inversion between *rrn* operons, IN(*rrnG-rrnE*).

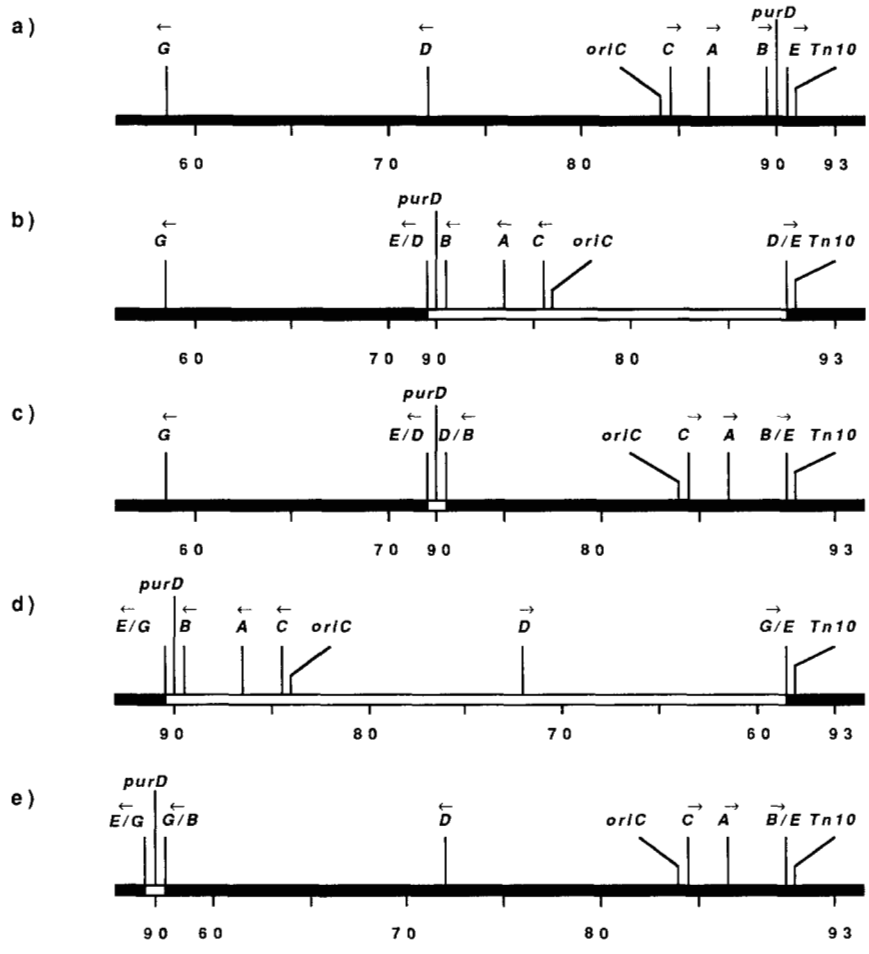

FIGURE 1.—Gene orders of wild-type *E. coli* K-12 and rearrangement mutants. The solid bars indicate DNA positioned normally, while the open bars indicate DNA that has been inverted or transposed. For clarity, the *rrn* operons are identified only by the letter which distinguishes the seven operons. The symbol *E/D* indicates a recombinant operon, *rrnE/D*, which contains the promoter of *rrnE* and the distal portion of *rrnD*. The polarities of the *rrn* operons are indicated by an arrow. a, The wild-type gene order is taken from the map compiled by BACHMANN (1983). The position of the *zja-1024*::Tn*10* insertion clockwise from *rrnE* is shown. b, The IN(*rrnD-rrnE*) inversion is present in the subline that includes W2637, W3110 and W3102 (HILL and HARNISH 1981; and our unpublished results). c, The Δ(*rrnB-rrnE*) Ω(*rrnD:rrnB-rrnE*) transposition mutation is present in CH1034. d, The IN(*rrnB-rrnE*) inversion is present in CH1326 and CH1351 constructed for this study. e, The Δ(*rrnB-rrnE*) Ω(*rrnG:rrnB-rrnE*) transposition mutation is present in CH1143. Mutants CH1034 and CH1143 have been described (HILL and HARNISH 1982).

MATERIALS AND METHODS

Bacterial strains and microbiological techniques: Isolation of mutants inverted between *rrnG* and *rrnE* will be described below. All other strains of *E. coli* K-12 have been described previously (HILL and HARNISH 1981, 1982), and relevant genetic structures are shown in Figure 1. Procedures for ultraviolet irradiation and for P1 transduction have been described (HILL and COMBRIATO 1973). LC medium was 1% Difco Bacto-tryptone, 0.5% Difco Yeast Extract, 0.3% glucose, and 0.5% NaCl and 0.0025 M $CaCl_2$.

DNA extraction and analysis: Techniques for DNA isolation, *Bam*HI and *Pst*I restriction endonuclease digestion, gel electrophoresis, transfer to diazotized paper and hybridization with [^{32}P]rRNA were as described previously (HILL and HARNISH 1981).

Construction of inversion mutants: The construction of the IN(*rrnG-rrnE*) inversion mutants followed the strategy described previously for the isolation of IN(*rrnD-rrnE*) and IN(*rrnD-rrnB*) mutants (HILL and HARNISH 1981), and this strategy is shown diagrammatically in Figure 2. The mutant used to start the construction was CH1135 which is a transposed duplication mutant with a duplicate copy of the *rrnB-rrnE* segment inserted into *rrnG* (HILL and HARNISH 1982); this transposed segment carries the missense suppressor *glyTsup* (Figure 2). Other relevant genotypic characters of CH1135 are that it is a derivative of Cavalli Hfr and it carries *trpA36*, a missense mutation suppressible by *glyTsup*. An essential feature of the chromosomal structure of CH1135 is that the two copies of the 44.5-kb *rrnB-rrnE* segment are oppositely oriented. Derivatives of CH1135 were selected which had spontaneously lost the suppressor, and these were screened for examples in which the section of the chromosome between the *rrnB-rrnE* segments had simultaneously become inverted. In order for this to have happened, the segments containing the *glyT* alleles must have paired and undergone a gene conversion accompanied by a reciprocal crossover. This screen yielded CH1318 and CH1319. Spontaneous Sup$^+$ mutants of CH1318 and CH1319 were selected and screened for examples in which the *glyTsup* mutation had occurred in the nontransposed *rrnB-rrnE* segment. This yielded CH1320 (from CH1318) and CH1321 (from CH1319). Suppressor loss was again selected, and this time the mutants were screened for examples in which the loss of suppression was through excision of the normal *rrnB-rrnE* segment. This screen produced CH1326 (from CH1321) and CH1351 (from CH1320). The structures of CH1326 and CH1351 were verified genetically by showing that in conjugation experiments, *lysA* was transferred from the Cavalli Hfr point of origin earlier and at much greater frequency than *argH*. It was verified molecularly by Southern analysis of a *Bam*HI digestion of DNA extracted from the mutants, using [^{32}P]rRNA as a probe (Figure 3). The *Bam*HI fragments containing *rrnG* and *rrnE* were missing in DNA from CH1351 (Figure 3, lane c), and they were replaced by two hybrid bands which can be identified as *rrnG/E* and *rrnE/G* from their sizes (LEHNER, HARVEY and HILL 1984).

Extended growth experiments: W3102 was grown in LC medium at 37° with aeration. Stationary phase cells were periodically diluted in such a manner that either 12 generations (cultures A, B, and C) or 20 generations (cultures D and E) were required to regain stationary phase. Cultures D and E were irradiated with a germicidal ultraviolet lamp

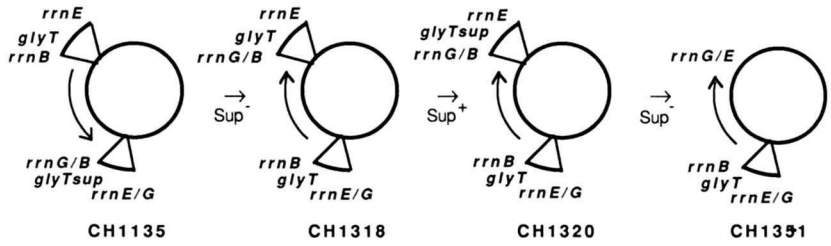

FIGURE 2.—Construction of mutants with an inversion between *rrnG* and *rrnE*. The construction used the same strategy described previously for the construction of IN(*rrnD-rrnE*) (HILL and HARNISH 1981) (see MATERIALS AND METHODS).

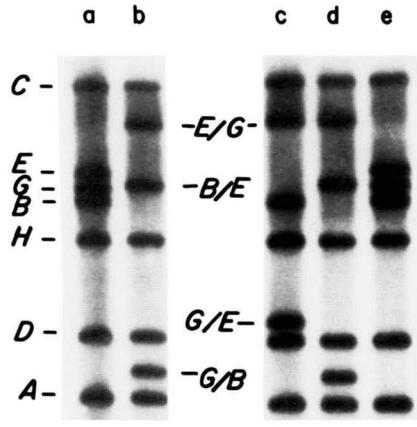

FIGURE 3.—*Bam*HI fragments coding for ribosomal RNA. Techniques for DNA isolation, *Bam*HI digestion, gel electrophoresis, transfer to diazotized paper, and hybridization with [^{32}P]rRNA were as specified in MATERIALS AND METHODS. Bands *A*, *B*, *C*, etc., correspond to genes *rrnA*, *rrnB*, *rrnC*, etc., while bands *E/G*, etc., correspond to recombinant operons *rrnE/G*, etc. DNA extracted from wild-type cells (lane a); CH1143 (lane b); CH1351 (lane c); revertants of CH1351 (lanes d and e). The bands from the normal *rrn* operons were used as internal standards (LEHNER, HARVEY and HILL 1984) for the measurement and identification of the recombinant ones.

before the passages began. The UV dose used was sufficient to reduce the viable cell count by 75%. Portions of selected passages were infected with P1 phage which had been grown on the *purD13 zja-1024*::Tn*10* donor, CH1045, tetracycline resistant transductants were selected, and these were tested for their Pur phenotype. The presence of the IN(*rrnD-rrnE*) inversion in the recipient culture reduced the Tet^R Pur$^-$ cotransduction frequency from 40% to 0.2%. Enhanced Pur$^-$ cotransduction was indicative of increasing proportions of wild-type cells relative to the inversion mutants. In control experiments, about five times more *total* Tet^R transductants were obtained with wild-type recipient cells than with the inversion mutants, presumably because of the greater uninterrupted flanking homology available for integration of the selected marker. Taking this factor into account along with the 40% cotransduction of Tet^R and Pur$^-$ normally obtained, a recipient population consisting of 1% wild type and 99% inversion mutants would yield roughly 2% Tet^R, Pur$^-$ cotransductants among the total Tet^R transductants. Portions of selected passages were adjusted to 8% dimethyl sulfoxide and frozen at −70°C. These frozen stocks were later thawed and used to inoculate cultures for the extraction of genomic DNA, and for the isolation of individual clones for further analysis.

RESULTS

Overgrowth of W3102 cultures by secondary inversion mutants: The large inversion carried in the *E. coli* K-12 subline including W2637, W3110 and W3102, imparts no deleterious effects that are apparent by simple inspection of clonal growth. When the same inversion was constructed in another genetic background, the mutants could be compared to the parent in a sensitive mixed growth protocol (HILL and HARNISH 1981). It was found that the inversion mutants grew about 1–3% less well. However, interpretation of this small difference was complicated by the fact that the mutant construction involved the introduction of a chromosomal segment from another strain, and the very small effect might have been due to lack of complete isogenicity rather than to the inversion. A much more critical test of the consequences of the inversion would be to compare directly the fitness of an inversion mutant to that of spontaneous wild-type revertants obtained from it. Since there was no way to simply select these wild-type revertants, we used the following approach.

If a wild-type configuration is more fit, extended growth of a large population of cells, all initially carrying the inversion, should result in the eventual overgrowth of the population by wild-type revertants. Wild-type revertants would be produced by an intrachromosomal recombinational event between oppositely oriented *rrn* genes as diagrammed in Figure 4 (type 1 crossover). To test this prediction, we chose to work with W3102. Other than the IN(*rrnD-rrnE*) inversion, it differs from K-12 only in that it is cured of the F$^+$ and λ episomes, and it carries the *galK2* mutation (BACHMANN 1973).

Two methods were used to detect the presence of wild-type cells within a population of inverted mutants. The first was a genetic procedure and involved the transduction of the population using P1 phage grown on a *purD13 zja-1024*::Tn*10* donor. The *purD* locus is closely linked to *rrnE*, within the inversion while Tn*10* is inserted on the other side of *rrnE*, outside of the inversion (compare Figure 1, a and b). Tn*10* and *purD* are cotransduced 40% when wild-type *E. coli* is used as recipient but only about 0.2% when the recipient carried IN(*rrnD-rrnE*). The low frequency of cotransduction found with the inversion recipient is due to the movement of *purD*$^+$ far from the site of the Tn*10* insertion.

The second method of detecting cells which have undergone a *rrn*-mediated rearrangement takes ad-

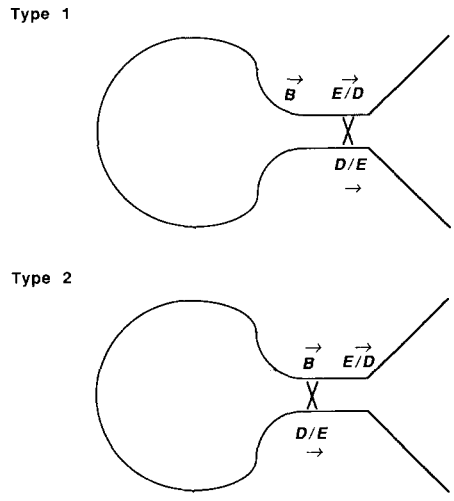

FIGURE 4.—Diagram illustrating two inversion events possible in W3102. Pairing and reciprocal recombination between *rrnE/D* and *rrnD/E* (type 1) converts the IN(*rrnD-rrnE*) inversion back to wild type. Pairing and reciprocal recombination between *rrnE/D* and *rrnB* (type 2) converts the IN(*rrnD-rrnE*) inversion to the Δ(*rrnB-rrnE*) Ω(*rrnD:rrnB-rrnE*) deletion-insertion structure (Figure 1).

vantage of the fact that none of the *rrn* genes contain either a *Bam*HI or a *Pst*I restriction endonuclease site. Digestion of total genomic DNA with *Bam*HI and *Pst*I followed by gel electrophoresis, Southern transfer, and probing with [^{32}P]rRNA produces a discrete and identifiable band for each of the seven *rrn* genes as well as for various hybrid *rrn* genes (BOROS, KISS and VENETIANER 1979; LEHNER, HARVEY and HILL 1984). This method has the additional advantage that it can detect any rearrangement caused by recombination between *rrn* genes, not just the one that converts IN(*rrnD-rrnE*) back to wild type. The genetic transduction method has the advantage of detecting wild type cells present at a low frequency in the population.

The extended growth experiments were conducted by serial passage as follows. At each passage, 1 μl of cells was diluted to 4.1 ml with LC medium and grown to stationary phase. Thus about 3×10^6 cells were passed and 12 generations were accumulated before the next passage. Periodically, portions of the cells were transduced, using P1 phage grown on the Tn*10* *purD* donor selecting for Tet^R and scoring Pur$^-$. The results of several clonally independent experiments are shown in Figure 5. For cultures A, B and C, the cotransducibility of *purD* with Tn*10* showed periodic increases, indicating that inversions back to wild type had occurred and that these wild-type cells were increasing their proportion within the population. However, the Tet^R, Pur$^-$ cotransduction frequency often declined upon further passing of the culture, indicating displacement of the wild-type cells by cells which still had the *purD* gene separated from the Tn*10* insertion site. For example, for culture A (Figure 5) a

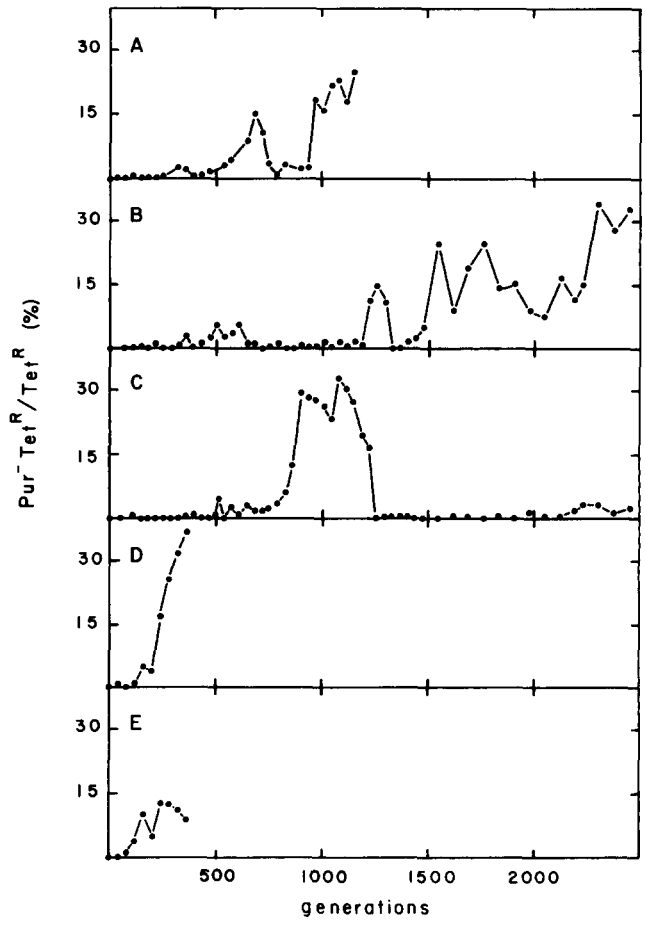

FIGURE 5.—Extended growth of W3102. Five clonally independent cultures of W3102 (A–E) were serially propagated as described in MATERIALS AND METHODS. Cultures D and E were irradiated with ultraviolet light before the passages were begun. Periodically portions of the cultures were infected with P1 phage grown on a *purD13 zja-1024*::Tn*10* donor so that appearance of cells with the normal linkage of these loci could be assessed (MATERIALS AND METHODS).

small but significant increase in Tet^R, Pur$^-$ cotransduction was observed after 324 generations, but by 396 generations it had fallen to the level observed with a fresh W3102 culture. The frequency again rose, peaking at 684 generations. At this point, streaking the culture for single colonies and testing them individually revealed that 2/8 gave 35–40% Tet^R, Pur$^-$ cotransduction while 6/8 gave less than 1% showing that wild-type revertants constituted roughly 25% of the population. Finally Tet^R, Pur$^-$ cotransduction reached even higher levels after 972 generations and remained high until the experiment was terminated after 1080 generations. At this point 7/8 single colonies derived from the culture were back inversions to wild type. Our interpretation of this sequence was that cells with the normal gene order have a definite advantage over cells with IN(*rrnD-rrnE*). However, other adaptations can occur which produce cells with a competitive advantage even

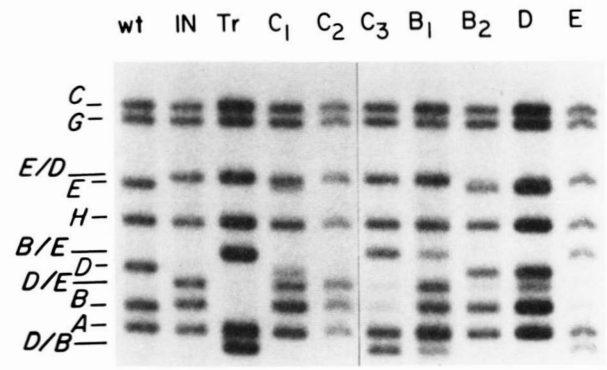

FIGURE 6.—*Bam*HI-*Pst*I fragments coding for ribosomal RNA. Techniques for DNA isolation, *Bam*HI and *Pst*I digestion, gel electrophoresis, transfer to diazotized paper, and hybridization with [^{32}P]rRNA were as described (HILL and HARNISH 1981). Conventions for the specification of bands on the autoradiograms was the same as in Figure 3. DNA extracted from wild-type cells (lane wt); W3102 (lane IN); CH1034 (lane Tr); generation 1080 (lane C_1), generation 1620 (lane C_2), or generation 2448 (lane C_3) of culture C; generation 1188 (lane B_1) or generation 2448 (lane B_2) of culture B; generation 360 of culture D (lane D); generation 360 of culture E (lane E). To prepare DNA from cultures B, C, D and E, frozen portions were used to inoculate fresh cultures which were used for the actual DNA extraction. The bands from the normal *rrn* operons were used as internal standards (LEHNER, HARVEY and HILL 1984) for the measurement and identification of the recombinant ones.

greater than the back inversion being measured. Since IN(*rrnD-rrnE*) cells were most abundant in the early stages, these additional adaptations generally occurred in cells still containing the original inversion, and they overgrew the culture, diluting out the "wild-type" revertant cells. Subsequently, within this *adapted* population, back inversions to wild type occurred again and produced another wave of Tet^R, PurD^- cotransduction. Such periodic selection is a well known phenomenon in the extended growth of cultures (ATWOOD, SCHNEIDER and RYAN 1951).

The course of overgrowth by more fit cell types was highly variable as shown by the clonally independent experiments B and C. For culture B, the Tet^R, Pur$^-$ cotransduction frequency increased and decreased throughout and was high when the experiment was terminated after 2448 generations. At the termination 4/4 single colonies derived from culture B gave 35–40% Tet^R, Pur$^-$ cotransduction, showing that the culture consisted primarily of back inversions to wild type. Culture C gave somewhat different results. Between 800 and 1200 generations, the Tet^R Pur$^-$ frequency was high and when sampled at 1008 generations, 6/12 single colonies were wild type. However, the Tet^R, Pur$^-$ cotransduction frequency fell abruptly at 1260 generations and never reached high levels again.

To further investigate the nature of cells present at various times in culture C, DNA was extracted from culture samples. These DNA preparations were digested with both *Bam*HI and *Pst*I, electrophoresed and probed with [^{32}P]rRNA (Figure 6). As suggested by the P1 transduction experiments, the sample prepared after 1080 generations (lane C_1) revealed bands diagnostic of IN(*rrnD-rrnE*) cells (bands D/E and E/D) as well as bands diagnostic of wild type cells (bands D and E). By 1620 generations (lane C_2), bands D and E had disappeared and the culture contained primarily IN(*rrnD-rrnE*) cells. After 2448 generations (lane C_3) new *rrn* bands unlike those produced by either IN(*rrnD-rrnE*) or wild-type cells were predominant. These bands were identical in size to those produced by CH1034 (lane Tr), a constructed mutant which has the *rrnB-rrnE* chromosomal segment deleted and transposed by insertion into *rrnD* (Figure 1c). Such a transposition mutant probably resulted from an inversion taking place in W3102 by a type 2 crossover as diagramed in Figure 4.

A similar analysis of DNA preparations from culture B confirmed that after 2448 generations (Figure 6, lane B_2), the banding pattern was that expected of a culture containing almost entirely wild-type cells with a small minority of IN(*rrnD-rrnE*) cells. Interestingly, after 1188 generations (lane B_1), DNA from culture B produced low but observable levels of bands the size of B/E, E/D and D/B. Likely culture B at this time contained a substantial minority of cells produced by the type 2 crossover (Figure 4), but this cell type disappeared and was not observed after 1332 generations (not shown).

Ultraviolet irradiation has been shown to increase recombination between *rrn* genes (HILL and COMBRIATO 1973). It was predicted, therefore, that UV irradiation of a W3102 culture should substantially increase the frequency of back inversions in the population and if these types were more fit, they should overgrow the population sooner than if their generation depended upon spontaneous rearrangement. Accordingly, two independent cultures of W3102 were irradiated with a dose of UV sufficient to reduce the viable cell count by 75%. After allowing the irradiated population to grow to stationary phase, it was passed as described for cultures A, B and C, except that greater dilutions were made at each passage, requiring 20 generations to reach stationary phase before the next passage. As anticipated, the cotransduction frequency of Pur$^-$ with Tet^R reached high levels sooner than had been observed without UV. Only about 120 generations were required for the frequency to reach levels significantly above those observed initially (cultures D and E, Figure 5). With culture D, the Tet^R, Pur$^-$ cotransduction frequency continued to increase through 360 generations. At this point, analysis of DNA extracted from the culture showed that wild-type cells constituted the large majority of the population (Figure 6, lane D). With culture E, however, the Tet^R, Pur$^-$ cotransduction frequency peaked at 240 generations and then declined. Analysis of DNA

extracted at 360 generations (Figure 6, lane E) showed that in culture E, Δ(*rrnB-rrnE*) Ω(*rrnD:rrnB-rrnE*) transposition mutants (produced by the type 2 crossover, Figure 4) had become predominant.

Even though the courses of the five experiments shown in Figure 5 were complex and variable, it is particularly significant that the frequency of cells with the original inversion had been severely reduced by the time each of the experiments was terminated. These experiments clearly demonstrated that the IN(*rrnD-rrnE*) inversion present in W3102 makes the cell less fit under these conditions of repeated transition from stationary to rapid growth. Back inversion to either the normal gene order or to the Δ(*rrnB-rrnE*) Ω(*rrnD:rrnB-rrnE*) structure, produces cells which have a competitive advantage over those with the IN(*rrnD-rrnE*) inversion.

Inversion of the *rrnG-rrnE* interval: In view of the small but significant effect of the inversion in W3102, we were interested in the consequences of a larger inversion and particularly one that was less symmetrical with respect to the replication origin. LOUARN *et al.* (1985) have described a very large inversion, IN(29–78), of essentially half of the chromosome. This inversion brought the replication terminus (or part of it) very close to the replication origin. This inversion was severely detrimental, causing the cell to be sensitive to rich media. An inversion between *rrnG* and *rrnE* would provide an intermediate case for comparison. IN(*rrnG-rrnE*) would include 31% of the chromosome, with the replication origin lying 6 min from one end and 25 min from the other. Therefore, we constructed mutants inverted between *rrnG* and *rrnE* (MATERIALS AND METHODS). It was immediately apparent that the inversion mutants (CH1326 and CH1351) were severely affected. They grew slowly on agar plates prepared from synthetic medium, producing goopy colonies. They had very low viability when streaked on broth plates, a phenotype much like that described for IN(29–78). In order to prove that the detrimental effects were due to the inversion, we isolated revertants. This was done by taking exponentially growing cultures of CH1326 and CH1351 in supplemented synthetic medium and diluting two clones of each into LC medium. The cultures were maintained in exponential growth by serial passage for about 25 generations by which time they reached stationary phase. The cultures were diluted and plated on plates containing synthetic medium. The majority of the colonies from three of the four clones were normal in appearance rather than goopy like the starting culture, suggesting that variants had overgrown these three cultures. A total of 11 colonies from these 3 cultures were characterized by Southern analysis of DNA extracts. Examples are shown in Figure 3. Of the 11, 9 had patterns characteristic of wild type (an example is shown in Figure 3, lane e), one retained that of the original inversion, and one had a pattern that was different from either the parental inversion mutant or from wild type. Its pattern (Figure 3, lane d) was identical to that of CH1143 (Figure 3, lane b) which is a transposition mutant Δ(*rrnB-rrnE*) Ω(*rrnG:rrnB-rrnE*) (Figure 1e). This latter revertant could have been produced by an event analogous to the type 2 crossover diagramed in Figure 4. These results proved that the severely detrimental effects exhibited by CH1326 and CH1351 were due to the IN(*rrnG-rrnE*) inversion and that they could be removed by reversion to wild type or by a different, compensating inversion.

DISCUSSION

Both inversion mutations studied here were detrimental to cell growth, the effects of IN(*rrnD-rrnE*) being slight, while those of IN(*rrnG-rrnE*) were severe. In both cases, reinversion to wild type or partial reinversion to a structure which left the *rrnB-rrnE* segment transposed relieved the effects. All of the genetic types depicted in Figure 1 have the same genetic content. The only differences are the gene order and the presence of recombinant *rrn* operons, and either of these differences could contribute to the detrimental effects. Therefore, a conceivable explanation of the disadvantages caused by the inversions would be that the recombinant *rrn* operons do not function optimally. Obviously reinversion to wild type could correct that defect. However, the enhanced fitness of the partial reinversions makes this explanation less satisfactory. These partial reinversions, which are effectively transpositions, do not restore either of the *rrn* operons altered by the original inversion. In fact, these mutants have a total of three rather than two recombinant *rrn* operons. We believe a more probable explanation is that the original inversion causes a displacement of genes outside the inversion relative to the replication origin which lies within the inverted segment (Figure 1). In the case of IN(*rrnG-rrnE*), genes to one side of the inversion would be 19 min closer and genes on the other side 19 min farther from the origin. For IN(*rrnD-rrnE*) the relative displacements are 6 min. This would have an effect on the average gene dosage for a chromosome replicating bidirectionally from a unique origin (CARO and BERG 1968; WOLF *et al.* 1968). Reinversion to the wild-type configuration fully corrects this situation. The reinversion caused by the type 2 crossover partially corrects the problem in that genes clockwise from *rrnE* are now only 0.9 min closer to *oriC* than normal while those counterclockwise from either *rrnD* or *rrnG* are only 0.9 min further away. This explanation accounts for the much greater severity of the effects of IN(*rrnG-rrnE*) or IN(29–78) (LOUARN *et al.* 1985)

when compared to IN(*rrnD-rrnE*) in that the displacement caused by the latter is much less extreme. Using hybridization techniques, DE MASSEY *et al.* (1987) have measured the gene dosages in the IN(29–78) mutant after a shift from minimal medium to broth. They found the relative gene dosages to be highly affected in this extreme case. SCHMID and ROTH (1987), studying mutants with *hisD* inserted at a variety of positions in the *S. typhimurium* chromosome, have shown that the expression level of the *hisD* gene is directly affected by the location of the gene, and that it correlates with the gene dosage gradient predicted for a chromosome replicating bidirectionally from a unique origin. Another effect of these inversions is that they alter the symmetry of the origin of replication relative to the terminus. Studies of integrative suppression of *dnaA* mutants have shown that this symmetry is important to cell growth (LOUARN, PATTE and LOUARN 1982). Based on these considerations, we would expect that very large inversions would not be deleterious if they were sufficiently symmetrical with respect to the replication origin. An inversion between *rrnG* and *rrnH* would be such a case since, although it includes half of the chromosome, it would be essentially symmetrical with respect to *oriC* (BACHMANN 1983). In fact, inversions between *rrnG* and *rrnH* have been isolated (Harvey and Hill, in preparation). The derivation of these mutants was complex, so they could not be compared directly to these considered here. Nevertheless, these IN(*rrnG-rrnH*) mutants grew well and did not have the obvious problems associated with IN(*rrnG-rrnE*).

Our identification of the IN(*rrnG-rrnE*) inversion mutants used in this study relied on Southern analysis to characterize the end points, and on interrupted mating to show that internal markers were actually present in inverted order (see MATERIALS AND METHODS). Initial characterization of the IN(*rrnD-rrnE*) inversion in W3102 and its relative W3110 relied on characterization of the end points alone (HILL and HARNISH 1981). The question could be raised as to whether the inverted segment has remained intact during the many years since its occurrence. In the case of W3110, it clearly has. A restriction map of the entire W3110 chromosome has been made (KOHARA, AKIYAMA and ISONO 1987), and the internal integrity of the IN(*rrnD-rrnE*) inversion was confirmed.

Perhaps one of the more interesting aspects of the serial transfer experiments described in Figure 5 was not the simple fact that IN(*rrnD-rrnE*) mutants were overgrown by revertants, but that this tendency persisted through many rounds of periodic selection. This suggested that even after IN(*rrnD-rrnE*) cells had acquired other mutations that made them more competitive under the experimental conditions, reinversion could still produce an additional growth advantage.

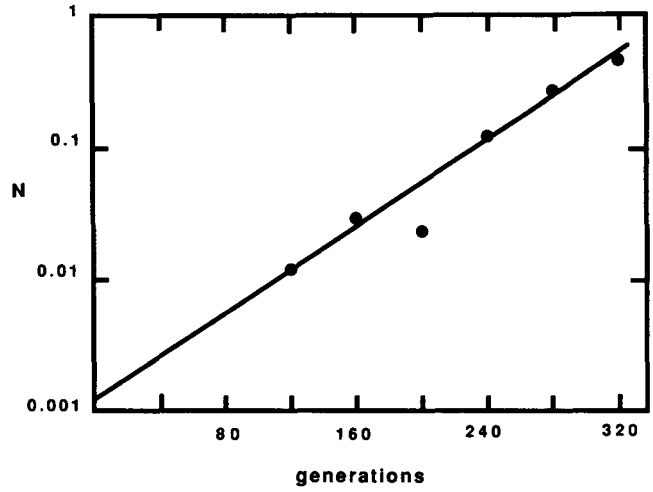

FIGURE 7.—Ratio of wild-type revertants to nonrevertants. The cotransduction frequencies obtained for culture D (Figure 5) were converted to the ratio of wild-type revertants to nonrevertants as described in MATERIALS AND METHODS. The log of this ratio (N) is plotted against the accumulated generations (g). The change in this ratio with culture growth is expected to be described by the relationship: $\ln N = \ln N_0 + \Delta k g \ln 2$, where Δk is the fractional difference between the growth constants of the two cell types.

From the serial transfer experiments (Figure 5), we can obtain some estimates of the frequency of inversion as well as the relative advantage the wild-type revertants have over the IN(*rrnD-rrnE*) mutants. As described in MATERIALS AND METHODS, the Tet^R, Pur^- cotransduction frequency can be used to estimate the relative proportions of revertant to nonrevertant cells in a culture. In the initial stages, the logarithm of the ratio of revertants to nonrevertants will increase linearly with a slope that is proportional to the difference between the growth constants of the cell types. (These calculations make no distinction between effects on actual growth as opposed to effects on how quickly the cells emerge from stationary phase after each serial transfer). In the case of the UV-irradiated culture D, we could plot the increasing fraction of wild-type cells to inversion cells through the first 320 generations and extrapolate back to the initial time (Figure 7). From this extrapolation, we estimate the frequency of wild-type revertants immediately after irradiation to have been 10^{-3}. The validity of this estimate depends on the assumption that the revertant cells recovered as rapidly from the irradiation as did the mutant cells. The slope of the line in Figure 7 indicates that the relative growth advantage (Δk) of the wild type was 2.7%. This agreed well with the 1–4% values obtained in earlier studies of constructed IN(*rrnD-rrnE*) mutants (HILL and HARNISH 1981). This value and data for cultures A, B and C from Figure 5 can in turn be used to estimate the spontaneous inversion frequency. The P1 transduction test can detect wild-type cells

only when they approach 1% of the population. For each of the three cultures, this took roughly 360 generations. If the wild-type cells had a growth advantage of 2.7%, they would increase their proportion by three logs in 360 generations. Thus the initial frequency of wild-type cells in the unirradiated cultures must have been roughly 10^{-5}. This is a 10–100-fold lower frequency than was found for the recombination between *rrnB* and *rrnE* leading to tandem duplications (HILL and COMBRIATO 1973; ANDERSON and ROTH 1978). Whether this difference in frequency is simply due to the greater distance separating the recombining partners, or whether other factors contribute is unclear. SCHMID and ROTH (1983) have noted that inversions are much rarer than might have been anticipated from other considerations. Even though inversions may occur less frequently than duplications, they are still more frequent than spontaneous base substitution. If these results can be extended to nature, they suggest that if an inversion, with as little effect on chromosomal organization as IN(*rrnD-rrnE*), occurred in a cell destined by chance to found a clone in nature, wild-type revertants (or partial revertants) would have a small but significant growth advantage. This would lead to displacement of the inversion mutants within a few hundred generations, even with the principle of periodic selection in operation.

This work was supported by U.S. Public Health Service Research grant GM16329 from the National Institutes of Health.

LITERATURE CITED

ANDERSON, R. P., and J. R. ROTH, 1978 Gene duplication in bacteria: alteration of gene dosage by sister chromosome exchanges. Cold Spring Harbor Symp. Quant. Biol. **43:** 1083–1087.

ATWOOD, K. C., L. K. SCHNEIDER and F. J. RYAN, 1951 Periodic selection in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **37:** 146–155.

BACHMANN, B. J., 1973 Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:** 525–557.

BACHMANN, B. J., 1983 Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47:** 180–230.

BOROS, I., A. KISS and P. VENETIANER, 1979 Physical map of the seven ribosomal RNA genes of *Escherichia coli.* Nucleic Acids Res. **6:** 1817–1830.

CARO, L. G., and C. M. BERG, 1968 Chromosome replication in some strains of *Escherichia coli* K-12. Cold Spring Harbor Symp. Quant. Biol. **33:** 559–573.

CASSE, F., M. PASCAL and M. CHIPPAUX, 1973 Comparison between the genetic maps of *Escherichia coli* and *Salmonella typhimurium*—length of the inverted segment in the *trp* region. Mol. Gen. Genet. **124:** 253–257.

DAVIDSON, N., R. C. DEONIER, S. HU and E. OHTSUBO, 1975 Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli.* X. Deoxyribonucleic acid sequence organization of F and of F-primes, and the sequences involved in Hfr formation. pp. 56–65. In: *Microbiology 1974,* Edited by D. SCHLESSINGER. American Society for Microbiology, Washington, D.C.

DE MASSY, B., S. BEJAR, J. LOUARN, J.-M. LOUARN and J.-P. BOUCHE, 1987 Inhibition of replication forks exiting the terminus region of the *Escherichia coli* chromosome occurs at two loci separated by 5 min. Proc. Natl. Acad. Sci. USA **84:** 1759–1763.

HILL, C. W., and G. COMBRIATO, 1973 Genetic duplications induced at very high frequency by ultraviolet irradiation in *Escherichia coli.* Mol. Gen. Genet. **127:** 197–214.

HILL, C. W., and B. W. HARNISH, 1981 Inversions between ribosomal RNA genes of *Escherichia coli.* Proc. Natl. Acad. Sci. USA **78:** 7069–7072

HILL, C. W., and B. W. HARNISH, 1982 Transposition of a chromosomal segment bounded by redundant rRNA genes into other rRNA genes in *Escherichia coli.* J. Bacteriol. **149:** 449–457.

HILL, C. W., R. W. GRAFSTROM, B. W. HARNISH and B. S. HILLMAN, 1977 Tandem duplications resulting from recombination between ribosomal RNA genes in *Escherichia coli.* J. Mol. Biol. **116:** 407–428.

KOHARA, Y., K. AKIYAMA and K. ISONO, 1987 The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:** 495–508.

LEHNER, A. F., S. HARVEY and C. W. HILL, 1984 Mapping and spacer identification of rRNA operons of *Salmonella typhimurium.* J. Bacteriol. **160:** 682–686.

LIN, REN-JANG, M. CAPAGE and C. W. HILL, 1984 A repetitive DNA sequence, *rhs,* responsible for duplications within the *Escherichia coli* K-12 chromosome. J. Mol. Biol. **177:** 1–18.

LOUARN, J., J. PATTE and J.-M. LOUARN, 1982 Suppression of *Escherichia coli dnaA46* mutations by integration of plasmid R100.1 derivatives: constraints imposed by the replication terminus. J. Bacteriol. **151:** 657–667.

LOUARN, J. M., J. P. BOUCHE, F. LEGENDRE, J. LOUARN and J. PATTE, 1985 Characterization and properties of very large inversions of the *E. coli* chromosome along the origin-to-terminus axis. Mol. Gen. Genet. **201:** 467–476.

RILEY, M., and A. ANILIONIS, 1978 Evolution of the bacterial genome. Annu. Rev. Microbiol. **32:** 519–560.

ROTH, J. R., and M. B. SCHMID, 1981 Arrangement and rearrangement of the bacterial chromosome. Stadler Genet. Symp. **13:** 53–70.

SANDERSON, K. E., 1976 Genetic relatedness in the family Enterobacteriaceae. Annu. Rev. Microbiol. **30:** 327–349.

SAVIC, D. J., S. P. ROMAC and S. D. EHRLICH, 1983 Inversion in the lactose region of *E. coli* K-12: inversion termini map within IS3 elements $\alpha_3\beta_3$ and $\beta_5\alpha_5$. J. Bacteriol. **155:** 943–946.

SCHMID, M. B., and J. R. ROTH, 1983 Genetic methods for analysis and manipulation of inversion mutations in bacteria. Genetics **105:** 517–537.

SCHMID, M. B., and J. R. ROTH, 1987 Gene location affects expression level in *Salmonella typhimurium.* J. Bacteriol. **169:** 2872–2875.

TIMMONS, M. S., M. LIEB and R. C. DEONIER, 1986 Recombination between IS5 elements: requirement for homology and recombination functions. Genetics **113:** 797–810.

WOLF, B., M. L. PATO, C. B. WARD and D. A. GLASER, 1968 On the origin and direction of replication of the *E. coli* chromosome. Cold Spring Harbor Symp. Quant. Biol. **33:** 575–584.

XIA, X.-M., and M. ENOMOTO, 1986 A naturally occurring large chromosomal inversion in *Escherichia coli* K12. Mol. Gen. Genet. **205:** 376–379.

Communicating editor: J. R. ROTH