Cloning of the Contiguous 165-Kilobase-Pair Region Around the Terminus of Escherichia coli K-12 DNA Replication

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Escherichia coli K-12 chromosomal DNA was partially digested with either EcoRI or HindIII, and cosmid libraries were constructed. By screening these libraries, a series of partially overlapping clones which covered the terC region was isolated. The cloned area spanned about 165 kilobase pairs, corresponding to the 29.7-to-33.2-min region of the genetic map of the E. coli chromosome. The 30-to-35-min region of the genetic map of the Escherichia coli chromosome appears to be genetically silent, and only a few genes have been mapped in this vast area. The bidirectional replication of the chromosome started at oriC (83.5 min) is known to meet in this region (14, 15). Recently, the replication terminus (terC) was mapped to 31.2 ± 0.2 min based on analysis of labeled restriction fragments in the synchronized replication cycle (4, 5). Thus, the correlation

FIG. 1. Genetic and physical maps of the isolated clones. The genetic scale (in minutes) is drawn at the top of figure, based on the data by Bouché et al. (5), and the positions of mapped genes are indicated. Maps of terC, trg, rimJ, and rac were taken from Bachmann (1), that of ksgD from Fouts and Barbour (7), and insertion sites of Tn10 in strains PLK1165 and PLK1273 from Bitner and Kuempel (3). The physical scale (in kb) was drawn from the terC locus (the left end of the 20.5-kb EcoRI fragment carrying trg), corresponding to the 241-kb position in the coordinate of Bouché (4). Under the physical scale, EcoRI and HindIII sites determined in this study and those reported by Bouché and Béjar (2, 4) are shown by vertical bars above and below the lines, respectively. Under these physical maps, the regions covered by isolated clones are indicated by bars. Clone names are given at the right side, omitting the common label pTER. Clones indicated by arrowheads are those shown in Fig. 2 and 3. EcoRI and HindIII sites on clones are indicated by broken and solid lines from the restriction maps. The position of the initial probe is shown by the open bar.

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of the genetic silence to the terC function has been postulated, and to clarify this question, attempts have been made to clone this area. Béjar and Bouché (2) have successfully cloned most of the terC region, but left three gaps in the 30-to-32-min region. In our attempt to correlate oriC function to terC function, we also noted that some restriction fragments generated from the terC area were difficult to clone with pBR322, probably because of the copy number effect. In this study, we first constructed cosmid libraries of the E. coli K-12 chromosome, from which a series of plasmids covering the terC region was isolated. The cosmid vector used was pH'C79 (13). In this vector DNA segments of up to 45 kilobase pairs (kb) can be cloned, and its copy number is known to be less than five (13).

E. coli C600 cells were lysed by the sodium dodecyl sulfate-lysozyme method, and after treatment with pronase the total DNA was prepared by phenol extraction, followed by treatment with RNase A. The DNA was partially digested with either EcoRI or HindIII and fractionated by sucrose-density-gradient centrifugation. The fraction containing 30- to 45-kb fragments was collected and ligated with the cosmids vector, pH'C79, which was linearized by using EcoRI or HindIII. The products were packaged with the lambda packaging system (9) and introduced into E. coli HB101, and the resulting colonies were screened by colony hybridization (10). The starting probe used was the trp-carrying PstI fragment (8.7 kb), which had been cloned into pBR322 by Harayama et al. (11). Plasmids were isolated from the clones hybridized to this probe. After construction of the restriction maps of the inserts, fragments generated from either the left or right part of the inserts were used as the next probe. By this chromosome-walking method, a large number of plasmids carrying partially overlapping inserts were isolated. The regions covered by the respective clones are shown in Fig. 1. Partial overlapping among the clones was confirmed by restriction analysis and cross-hybridization. Figures 2 and 3 show the results obtained with seven representative clones (indicated by arrowheads in Fig. 1). In lanes 1 through 3 of Fig. 3, for example, digests of pTERL103, pTERK65, and pTERe3 were resolved by gel electrophoresis and hybridized with 32P-labeled pTERK65. It is clear that these clones overlap each other at different portions of the plasmids. Overlapping fragments identified in this way are indicated by dotted lines in Fig. 2. It should be noted that clones carrying inserts at different cloning sites generate different sizes of partially overlapping fragments unless common fragments are generated from the overlapped region. The entire region covered by these clones spans about 165 kb in length and corresponds to the 29.7- to 33.2-min region of the genetic map when the position of the starting trp probe was fixed at 31.1 min (1; Fig. 1). These restriction maps were compared with those of Bouché (4), which had been constructed by a different procedure. The maps coincided well, except for the region between 328 and 337 kb (Bouché coordinates). The maps of this part were inverted, as reported by Béjar and Bouché (2). In Fig. 1 our maps are shown in comparison with the corrected Bouché maps.

A series of transposon insertion mutants have been isolated from the terC area (3, 8). To correlate the isolated clones to the genetic map, we examined the insertion sites on four mutant strains: KF1053 carrying Tn5 and Tn10 at 29.8 and 29.7 min (8), PLK1165 carrying Tn10 at 31.1 min (3), PLK1273 carrying Tn10 at 32.3 min (3), and KF1127 carrying Tn10 at 34.2 min (8). Since Tn10 has a single EcoRI site (6), the fragment carrying the Tn10 insertion should be split by EcoRI into two fragments, each linked to the 3.1- and 6.4-kb moieties of Tn10. Chromosomal DNAs from mutant and C600 cells were digested by EcoRI, resolved by gel electrophoresis, and hybridized with the respective 32P-labeled clones. As expected, the insertion sites in strains PLK1165

![Fig. 2](image-url)  
**Fig. 2.** The restriction patterns of isolated clones resolved by agarose gel electrophoresis. Enzymes used for digestion are given above the lanes, and fragments generated from overlapped regions are connected by dotted lines. Sizes (in kb) and positions of HindIII-digested λDNA are indicated at the left side, and the names of plasmids are given under the lanes. The 6.7-kb fragment seen in lanes 1 to 5 and lanes 9 and 10 is the vector DNA. Vector-containing fragments in lanes 6, 7, and 8 are indicated by arrowheads.

![Fig. 3](image-url)  
**Fig. 3.** Hybridization analysis of isolated clones. EcoRI digests of isolated clones and chromosomal DNAs of C600, PLK1165, and PLK1273 cells were resolved by agarose gel electrophoresis, transferred to nitrocellulose filters, and hybridized with 32P-labeled pTERK65 (lanes 1 to 3), pTERR104 (lanes 4, 5, 6, 10, and 11), and pTERR210 (lanes 7, 8, 9, 12, and 13). Sizes (in kb) and positions of HindIII-digested λDNA are indicated at the left side, and the sources of DNA are given under the lanes. The 6.7-kb fragment seen in lanes 1 through 7 is the vector DNA.
(31.1 min) and PLK1273 (32.3 min) were detected in the corresponding regions (lane 10 through 13, Fig. 3), and those in strains KF1053 (29.7 to 29.8 min) and KF1127 (34.5 min) located near the end or outside of the cloned area were not identified (data not shown). It should also be mentioned that the Tn5 insertion in strain KF1298 (8) was identified at the 31.9-min position (data not shown).

As mentioned previously, only a few nonessential genes have been shown to reside in the vicinity of the terC region. At present, little is known about why this large region is genetically silent. It is possible that this terC area has an important function in the termination of chromosomal replication, so that no gene resides in this area. According to Henson et al. (12), however, deletion of the 30-to-31.4-min region flanking terC causes no apparent effect on cell growth. Another possibility to consider is whether nonessential genes which do not lead to easily detected mutant phenotypes have been enriched in this region. The plasmids isolated in this study would be useful for elucidating the function of this region.

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LITERATURE CITED