Deletion of the terminus region (340 kilobase pairs of DNA) from the chromosome of Escherichia coli

(replication inhibition/cell cycle events/DNA hybridization/SOS induction)

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ABSTRACT  A strain of Escherichia coli with a 7-minute (340 kilobase pairs of DNA) deletion of the terminus region of the chromosome was isolated. This deletion was probably an IS10-promoted event and its extent was characterized by both genetic and DNA hybridization analyses. The most dramatic property of strains harboring this deletion was the absence of the sites that inhibit clockwise- and counterclockwise-traveling replication forks. These strains also grew slowly, produced many nonviable cells, were filamentous, and appeared to have an induced SOS system.

The terminus region of the chromosome of Escherichia coli is located between min 29 and min 35 on the genetic map (1). It is directly opposite the origin of replication on the circular chromosome, and bidirectional replication forks that initiate at the replication origin meet in the terminus region at the end of the replication cycle (2). One striking property of the terminus is that both clockwise- and counterclockwise-traveling replication forks are inhibited as they proceed across it (3-6). Except for the inhibition of replication, very little is known concerning this region of ~250 kilobase pairs (kb) of DNA. It seems likely that functions important in events at the end of the replication cycle are among those encoded here (7). Some examples include the separation of catenated daughter chromosomes, partitioning of daughter chromosomes to daughter cells, and coordination of chromosome replication with cell division. It is even possible that the sites that inhibit replication are an important component of at least some of these processes.

Our approach to studying the functions encoded in the terminus has been to obtain strains with deletions in this part of the chromosome. Analyses of such strains should demonstrate if any DNA of this region is essential. In addition, it should be possible to determine if aberrant cell cycle phenotypes correlate with particular deletions. This type of analysis has been made possible by the isolation of various transposon insertions (8, 9), which can be used to obtain deletions (10). Furthermore, a restriction map has been constructed for the terminus region (11), and this greatly facilitates characterization of deletions.

Previously, we isolated strains in which large segments of the terminus were deleted, but there were no obvious phenotypic effects (12, 13). In this report, we describe the isolation and properties of a strain containing a larger deletion that removed the entire terminus region (~7 min or 340 kb). We demonstrate that strains harboring this deletion had an abnormal phenotype, which included the absence of sites that inhibit replication forks.

MATERIALS AND METHODS

Isolation of Strain PLK1608. Strain PLK1608, the original isolate that contained the large deletion of the terminus region, was obtained as the result of the following series of steps. The isolation started with strain PLK1415, which was a λ reverse c1857S7 (λrev) lysogen that we described previously (13). Bacteriophage P1 was used to transduce sci-233::Tn10 into this strain. This transposon was inserted at min 28.9 (unpublished experiments), which was near the left end of the λrev integrated at min 29.7 (Fig. 1). The tetracycline-resistant (Tc') strain (PLK1417) was then plated on fusaric acid plates (14) to obtain tetracycline-sensitive (Tc') derivatives. These derivatives usually result from deletions that initiate at the inside end of one of the IS10s of Tn10 and extend through the gene conferring Tc' (10). Our rationale was to obtain deletions that extended to the right into λrev, removing the left end of the prophage. Strain PLK1422 was obtained from these Tc' derivatives, and it was temperature sensitive and lysed at 42°C due to the remaining λrev genes, but it produced no effective phage particles. Subsequent analysis by DNA-DNA hybridization demonstrated that the left end of the prophage was deleted, the internal HindIII fragment of Tn10 was absent, and the strain still contained IS10 DNA.

When strain PLK1422 was plated at 42°C, ~10^6 cells formed colonies. Since the λrev could not undergo its normal excision, some of these temperature-resistant derivatives were expected to result from further IS10-promoted deletions that removed the remaining λrev DNA and extended further into the terminus region. We obtained a number (~1000) of temperature-resistant isolates and screened them for loss of genetic markers in the terminus region. One of these strains was both Sad' and Man', and it (PLK1608) was subjected to further analysis.

Other Bacterial Strains and Media. Bacteriophage P1 transduction (15) was used to transduce the large deletion (called Δ5) into other strains, including PLK504 and PLK583. These strains were dnaAts and had a P2sigs prophage integrated near metG and galK, respectively (3, 4). Strains harboring plasmids pBKpaBB', pBKmanaA', pBKaroD', pJH12, pJH106, pJH113, and pJH142 were isolated in our laboratory (ref. 12; unpublished experiments). Plasmid pDK07 (pyrF') was constructed by Donovan and Kushner (16); pTH51 (try'), by Harayama et al. (17); pGS27, by Shaw and Guest (18); and pMU352 (try'), by Cornish et al. (19). Plasmids pLC4-6 (try') and pLC-dgs-4 (dgsA') were from the Clarke and Carbon collection (20).

Strains were grown in LB (rich) or M9 (minimal) medium (15). LB plates containing 20 μg of tetracycline per ml were used for selecting and scoring Tc'. Minimal plates containing the following supplements were used for scoring other loci: 5 mM succinic semialdehyde for sad (21), 20 μg of m-fluorotyrosine per ml for tyrR (22), glycerol plus fumarate (GF) in anaerobic conditions for nirR (23), and 40 mM fumarate for fumA (24). Swarm plates were used for scoring trg (25).

Abbreviations: kb, kilobase pair(s); Tc', tetracycline-resistant; Tc', tetracycline-sensitive.

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McConkey medium containing mannose was used for scoring manA (15), and hydrolysis of p-nitrophenyl-β-D-glu-curonide was used for scoring uidA (26).

**DNA Preparation and Hybridization.** Procedures for purification of chromosomal and plasmid DNA, DNA digestion, gel electrophoresis, and autoradiography were described previously (12). Plasmid DNA was nick-translated (27) and hybridized with chromosomal DNA in dried agarose gels (28).

Hybridizations to episome DNA attached to nitrocellulose filters were performed according to Kuempel et al. (3), except that nick-translated, chromosomal [32P]DNA was added as an internal standard instead of [14C]DNA. This DNA was isolated from stationary-phase cells in which all genes were present at equal frequency. Hybridizations to plasmid DNA were conducted by similar procedures, except that 0.5 μg of DNA was attached to each filter, and the hybridization solution contained 20 μg of salmon sperm DNA per ml. The plasmid DNA was boiled for 90 sec in 0.2 M NaOH before attachment to the filters. This was done to convert the supercoiled plasmid DNA to a linear, single-stranded form.

**RESULTS**

The isolation of strain PLK1608, which contained Δ5, is described in Materials and Methods. Its properties indicated that Δ5 started at an IS108 remaining from zci-233::Tn10, extended through λrev, and ended on the other side of the terminus (Fig. 1). Genetic analysis of this strain demonstrated that loci between min 28.9 and min 35.8 were absent. The strain was tyrR (min 29.2), nirR (min 29.4), trg (min 31.4), sad (min 34), fumA (min 35.6), manA (min 35.7), and uidA (min 35.8). It seemed likely that the deletion ended just before tyrS (min 36), which is an essential locus (29). Strain PLK1608 was also pyrF+ (min 28.3).

This cluster of altered loci could be transduced into other strains. In order to have a selectable marker flanking the deleted region, zdg-232::Tn10 (min 36.2; unpublished experiments) was introduced into PLK1608, and this strain was the donor in subsequent transductions. Bacteriophage P1 grown on this strain was used to transduce Te+ into other strains, and the frequency of cotransduction of manA with Te+ was usually about 1%. When these strains were tested for other markers, they were invariably (75/75) tyrR nirR trg sad fumA uidA. This indicated that no substantial amount of DNA remained between zdg-232::Tn10 and tyrR. If such a segment was still present, it would be expected that recombination in that segment would sometimes give Te+ manA recombinants that were tyrR+. Since tyrR and the Tn10 insertion are located at min 29.2 and min 36.2, respectively, this was a cotransduction of loci that are separated by 7 min on the genetic map. Bacteriophage P1 normally does not exhibit cotransduction of loci that are separated by more than ~2 min (1).

The results of DNA-DNA hybridizations were consistent with the above genetic observations and provided further evidence that Δ5 removed the entire terminus region. To conduct these analyses, a collection of plasmids and bacteriophage containing DNA fragments from the terminus region was assembled (Fig. 1). These DNA probes were pMU352, pGS27, λrev, pJH142, pTH51, pJHI66, pJH112, pJHI13, pLCH-dgs-4, and pBKmanA. Individual DNAs were nick-translated, and [32P]DNA was then hybridized with DNA from wild-type and Δ5 strains in dried agarose gels (28). In each case, the Δ5 strain was missing terminus region DNA that was present in the wild-type strain. Fig. 2 shows the results of some typical hybridizations. The faint band in Δ5 DNA in lane 4 contained IS10 sequences; the probe used in this hybridization, pHI12, contained an IS10 element (12). Presumably, the IS10-containing fragment in Δ5 DNA was the fusion fragment formed as a result of the deletion. When λrev was used as the probe (lanes 5 and 6), several bands appeared in DNA of the wild-type rac+ strain used. The 43-kb fragment contained DNA from the lac-defective prophage at min 34.5 (6), the 6.7- and 25-kb fragments contained DNA from the rac-defective prophage at min 29.7 (6), and the 4.8- and 10.3-kb fragments were from the qsr'-defective prophage, which is located near the gal locus at min 16 (30, 31). Only the 4.8- and 10.3-kb fragments remained in the strain containing Δ5.

Since all of the terminus region appeared to be removed by Δ5, it was expected that this strain would exhibit an altered phenotype, if the terminus region contains any functions important for growth in laboratory medium. Indeed, strains containing Δ5 were grossly abnormal. Examination of cultures demonstrated that many filamentous cells and mini-
cells were present as well as some cells with normal morphology. In addition, the cells had a reduced growth rate, growing at about one-half the rate of parental cells in minimal and rich medium at 37°C. Also, only about one-half of the cells formed colonies.

One of the most dramatic alterations that was observed in strains harboring Δ5 was that deletion of the terminus region removed the sites that inhibit replication forks. To test for the presence or absence of this inhibition, Δ5 was transduced into strain PLK583, which contains a temperature-sensitive dnaA mutation and a temperature-inducible P2sig5 prophage integrated at min 16. This prophage can be used to initiate replication forks from a point near the terminus region. As in our previous studies (4), exponentially growing cells were first incubated at 28°C for 170 min in medium lacking required amino acids. This allowed completion of existing replication cycles and halted initiation of new cycles. The cells were then shifted to 42°C and the amino acids and [3H]thymine were added to the medium. Initiation of normal replication cycles did not occur, due to the temperature-sensitive dnaA mutation. Replication cycles were initiated at the integrated P2sig5 prophage, because its temperature-sensitive repressor was inactivated. This prophage excised itself poorly from the chromosome, and replication forks initiated at the prophage proceeded in both the clockwise and counterclockwise directions into the bacterial chromosome (ref. 4 and Figs. 3 and 4).

[3H]DNA from induced cells was first hybridized to a variety of episome DNAs (Fig. 3). This was done to determine on a large scale where replication was occurring in the chromosome. When P2sig5 integrated at min 16 was used to initiate the replication cycles in a strain containing Δ5, the counterclockwise-traveling replication forks (right to left in Fig. 3) proceeded around the chromosome. This was demonstrated by the decreasing amounts of hybridization to the F'152, F'1ac, F'101, F'111, and F'116 episome DNAs. The amount of hybridization to the F'129 DNA was considerably increased, however. This was presumably caused by the clockwise-traveling replication forks, which proceeded through the terminus region and then replicated the F'129 and F'116 regions of the chromosome. This is distinctly different from the results obtained from strain PLK583, which contained the terminus region that inhibited replication forks (4). Data obtained with this strain are shown as a dashed line in Fig. 3.

In order to determine more accurately what happened to clockwise-traveling replication forks, hybridizations were also conducted using various cloned fragments that flank the terminus region. Fig. 4 shows that there was no inhibition of replication forks as they proceeded from the trp (min 27.7) and pyrF (min 28.3) loci into the region containing the aroD (min 37.1) and pabB (min 39.8) loci. This was in contrast to the results obtained with strain PLK583, in which virtually none of the forks that replicated trp and rac (min 29.7) proceeded into the region containing man and aroD (4). These data are shown as a dashed line in Fig. 4.

We also transduced Δ5 into strain PLK504, in which P2sig5 was inserted at min 46. This permitted a determination of whether Δ5 also removed the sites that inhibit counterclockwise-traveling replication forks. [3H]DNA from induced cells was hybridized to the cloned fragments that flank the terminus region (Fig. 5). Contrary to results obtained with strain PLK504 (dashed line in Fig. 5), counterclockwise-traveling replication forks were not inhibited.

**DISCUSSION**

As part of our approach to identifying functions encoded in the terminus, we have been isolating increasingly larger deletions. The rationale has been that some loci important for
cell cycle phenomena are probably encoded in the terminus, and an analysis of large deletions would eventually identify the location of these sites and the phenotypes associated with their removal. These regions could then be studied further, using either smaller deletions or reininsertion of fragments from the deleted region. An analysis of deletions would also demonstrate if any of this DNA is essential. Previous deletion strains that we studied had no obviously altered phenotype. This included deletion of the interval from kb 289 to kb 330 (ref. 12; see Fig. 1), the interval from kb 168 to kb 247 (13), and the interval from kb 165 to kb 289 (unpublished experiments). Strain PLK1608 (Δ5) was isolated in an attempt to obtain strains containing still larger deletions, which had altered phenotypes.

Results reported here indicate that the deletion in strain PLK1608 (Δ5) removed the entire terminus region. One set of observations that indicated that the terminus region was deleted was the genetic properties of this strain. All of the loci that were tested, in the interval from tyrR through uidA, were mutant in the deletion strain. In addition, results of transductions with bacteriophage P1 also indicated that the deletion extended from tyrR through uidA, for it was possible to transduce all of the properties associated with Δ5 into other cells.

DNA-DNA hybridization experiments provided further evidence that the region from tyrR through uidA was deleted. DNA of strains harboring Δ5 was hybridized with [32P]DNA probes containing a number of different fragments from the terminus region (Fig. 1). The Δ5 strain was missing all of the terminus region DNA tested (Fig. 2 and unpublished results). Ultimately, to be certain that all of the terminus DNA was deleted, it will be necessary to probe with fragments corresponding to the entire region and to identify the junction fragment that contains DNA from both sides of the terminus region and the IS10 that links them.

In addition to removing the genetic loci and DNA regions described above, Δ5 also removed the sites in the terminus region that inhibit replication forks. Clockwise-traveling replication forks were severely inhibited in the terminus region in strains containing a normal terminus region (4), and this inhibition was completely absent in a strain harboring Δ5 (Figs. 3 and 4). The sites that inhibit counterclockwise-traveling replication forks were also removed by Δ5 (Fig. 5).

If the terminus region and/or the sites that inhibit replication forks provide functions that are important for events at the end of the replication cycle, strains harboring Δ5 should have an altered phenotype. Consistent with this, these strains grew at a reduced rate compared to otherwise isogenic strains, and only one-half of the cells formed colonies. The cell size was quite heterogeneous, ranging from the size of minicells up to filaments over 60 μm long. Preliminary autoradiographic experiments indicated that a number of cells lacked DNA. These properties suggest that the strains were affected with respect to the separation of catenated daughter chromosomes, partitioning of chromosomes to daughter cells, or coordination of chromosome replication with cell division.

The excessive filamentation of the cells appeared to be caused by induction of the SOS system. When this system is turned on, recA protein is activated and lexA repressor is degraded (32). Consequent derepression of the sfiA gene inhibits cell division. Several observations indicated that the SOS system was induced in strains containing Δ5. For example, when Δ5 was transduced into a strain containing sfiA-::MudIlac, β-galactosidase was expressed, even in the absence of external DNA-damaging agents. Another indication that induction of the SOS system was responsible for filamentation in Δ5 strains was that filaments did not form when recA56 was present. These strains still grew slowly, however, which indicates that SOS induction was not responsible
for all of the properties of Δ5 strains.

In summary, experiments reported here demonstrate that the terminus region, including the sites that inhibit replication forks, can be deleted from the chromosome. Even though strains harboring Δ5 were viable, they had a dramatically altered phenotype that was not observed in strains harboring smaller deletions. This phenotype could be the result of loss of the sites that inhibit replication forks, loss of other sites that are important in cell cycle phenomena, or lack of proper coordination among these functions. Part of the aberrant phenotype appeared to be due to induction of the SOS system. Further experiments are necessary to determine the basis of these phenotypic alterations, whether insertion of various DNA fragments reverses the alterations, the cause of SOS induction, and which alterations are due to SOS activation.

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