JOURNAL OF BACTERIOLOGY, Sept. 1988, p. 4293–4298
0021-9193/88/094293-06$02.00/0

Vol. 170, No. 9

Location of Sites That Inhibit Progression of Replication Forks in the Terminus Region of *Escherichia coli*

ANTHONY J. PELLETIER, THOMAS M. HILL, AND PETER L. KUEMPEL*

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347

Received 9 February 1988/Accepted 10 June 1988

We used a Southern hybridization assay to locate precisely the sites at which DNA replication is arrested in the terminus region of the *Escherichia coli* chromosome. The assay was based on the properties of restriction fragments that contain stalled replication forks. Replication forks that entered the terminus from the clockwise direction with respect to the genetic map were inhibited near *manA* at a site called T2, which we located at kilobase 442 on the physical map of Bouché (J. P. Bouché, J. Mol. Biol. 154:1–20, 1982). Those that entered the terminus region traveling in the counterclockwise direction were inhibited near *pyrF* at a site called T1, which we located at kilobase 90. In each case we found only a single, precise site of arrest. Inhibition at T1 was not detectable in our assay in strains lacking the *trans*-acting locus *tus*, which is located near T2 and is required for T1 to function. We demonstrated that the sites of inhibition are also used during termination of replication in exponentially growing, wild-type cells. In all previous studies on the terminus of *E. coli*, inhibition has only been detected in strains that were modified so that the origin used was placed near the terminus to force the use of the sites of inhibition.

Termination of DNA replication and correct resolution of the daughter molecules is a critical aspect of the overall process of chromosome replication. This aspect of replication is still poorly understood. One common theme that has emerged is the occurrence of specific sites at which DNA replication is inhibited. There are several examples of such sites in bacterial systems, including the plasmid R6K (3), the chromosome of *Bacillus subtilis* (16), and the chromosome of *Escherichia coli* (12, 13). In all of these systems, there is a terminus region that is known to inhibit replication, thereby ensuring that termination and resolution occur in that region. In addition to these bacterial systems, inhibition sites have been identified recently in the DNA flanking an intergrated copy of polyomavirus in cultured rat cells (2) and in chromosomal replication in pea plants (7a).

In previous experiments it has been demonstrated that in *E. coli*, replication forks are severely inhibited as they pass through the terminus region between *trp* (minute 27.7) and *manA* (minute 35.7), which are approximately opposite the origin of replication (12, 13). A physical and genetic map of this region is presented in Fig. 1 (1, 4).

Recently, it has become apparent that termination is more complex than originally thought. The terminus region actually contains two separate regions, referred to as T1 and T2, that inhibit replication forks and that are located at either edge of the terminus region (5, 9). These results were obtained by assays based on DNA-DNA hybridization to determine gene frequencies (5, 9). These assays, to which we refer as marker-frequency assays, were used to monitor replication from inducible origins integrated near the terminus. Replication forks traveling clockwise with respect to the genetic map pass through T1 and are inhibited in the T2 region between minutes 33.5 and 35.5. In an analogous fashion, counterclockwise-traveling forks pass through T2 and are inhibited in the T1 region between minutes 28 and 29. Thus, inhibition is polar. Most recently, a *trans*-acting factor called *tus* (for terminus utilization substance) has been identified by deletion analysis (10). The gene for this factor is at or near T2 and is required for T1 to function. It is not known whether *tus* is also required for T2 to function.

An important distinction must be made between the sites at which inhibition occurs (the sites of arrest) and the loci required for inhibition. The latter can be determined by mutational analysis. Hill et al. (10) have used deletion analysis coupled with the marker-frequency assay to determine that the DNA required for inhibition at T2 is in a 4-kilobase (kb) region between kb 438 and 442. Since this interval also includes *tus*, such deletions also abolish inhibition at T1. Deletions of T1 have not yet been obtained, so the existence of a required locus at T1 has not been demonstrated. From deletions that do not affect T1, however, it is known that any required sequences must reside to the left of kb 100.

Although such deletion experiments can determine the location of required loci, they cannot demonstrate where inhibition occurs. The marker-frequency assay was sufficient to allow determination of the region in which inhibition occurs. However, in a typical experiment, the region through which the effects of inhibition were observed spanned 50 kb or more (9, 10). From these data, inhibition seemed to take place throughout the 50-kb region in which the effects were observed.

In order to locate accurately the sites of arrest in the T1 and T2 regions and to determine the nature of the apparent upstream inhibition, we developed an assay that is far more sensitive than the marker-frequency assay. Weiss and Wake (15) have used a gel system that was designed to detect the presence of replication forks in a given region of DNA to locate the site of inhibition in the terminus of *B. subtilis*. We developed a similar assay and used it to determine accurately the location of the sites of arrest at T1 and T2. The replication forks were arrested at two precise sites, one in the T1 region and one in the T2 region. We demonstrated further that these sites are used in replication cycles that are initiated from *oriC* during exponential growth. This demonstrates that the use of the sites is not confined to situations in

* Corresponding author.

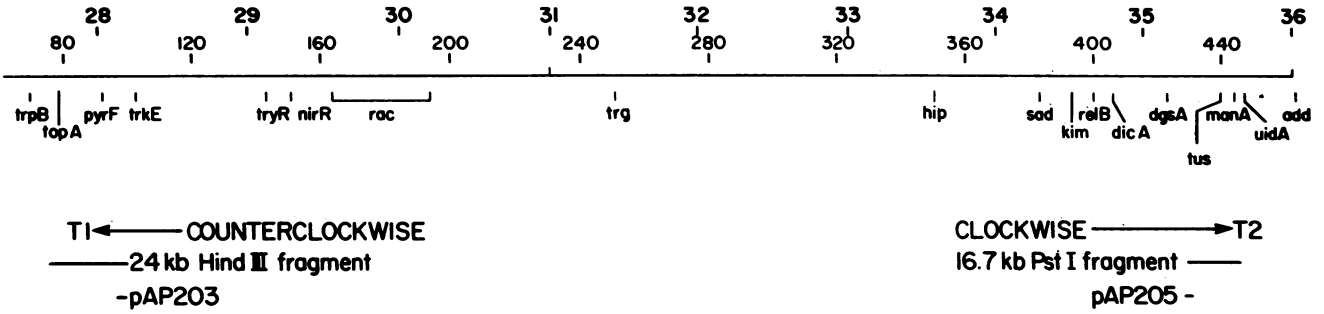

FIG. 1. Map of the terminus region showing genetic (1) and physical (4) coordinates. T1 inhibits counterclockwise-traveling replication forks; pAP203 was used as the probe to locate T1 in the 24-kb *Hin*dIII fragment (kb 75 to 99). T2 inhibits clockwise-traveling replication forks; pAP205 was used as the probe to locate T2 in the 16.7-kb *Pst*I fragment (kb 429 to 446).

which the origin and terminus are asymmetrically placed with respect to each other.

MATERIALS AND METHODS

Bacterial strains and plasmid. Strains PK998 and PK1012 have been described elsewhere (9). Strain PK2340 is a derivative of PK1012 that contains Δ2337 (deletion of kb 433 to 443) (10). Plasmid pPK1009 is a low-copy-number cosmid containing chromosomal DNA from kb 433 to 443 in cosmid pRI40 (10). Strain PB153 is an Hfr strain, as are the derived inversion strains CH1027 and CH1130 (8) and CH1351 (Charles Hill, personal communication).

The procedure used to induce replication from the prophage origin has been described in detail elsewhere (9). Minimal (M9; supplemented with the required amino acids and thymine) and rich (LB) media were made as described by Maniatis et al. (14).

Southern hybridization assay. The DNA to be tested was prepared as described by Maniatis et al. (14) for harvesting chromosomal DNA. For alkaline gels, this DNA was digested directly with either *Pst*I or *Hin*dIII (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and then denatured at pH 11 at 65°C and fractionated on an alkaline agarose gel (14). For neutral gels, the DNA was first sheared by vortexing it for 3 min and then was digested with the appropriate enzyme and fractionated on 0.5% agarose gels in TBE buffer (14). DNA was transferred in situ to a nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, N.H.) and hybridized to a nick-translated DNA probe by the procedures described by Maniatis et al. (14).

Testing for reversion of inversions. Chromosomal DNA was digested to completion with *Bam*HI and *Pst*I, fractionated on 0.5% agarose, transferred to nitrocellulose, and hybridized with a nick-translated probe derived from cloned ribosomal DNA (pNO2680) (6). Since neither of the enzymes cuts in the ribosomal repeats, each *rrn* gene migrated as a discrete band. The pattern for each strain was compared with the published pattern (8).

RESULTS

Identification of sites of inhibition. The rationale of the assay was as follows. A restriction fragment that contains a site of inhibition would contain a stalled replication fork if that site was in use. This would presumably be a Y structure, as suggested by Weiss and Wake (15). The location of the site of arrest was deduced from the length of the newly synthesized strands. The most straightforward way to determine this was by denaturing the DNA. The leading strand, at least, should be present as a continuous strand extending from one end of the restriction fragment (the end proximal to the origin of replication) to the point at which replication stopped. The DNA was fractionated on alkaline agarose gels, transferred to nitrocellulose, and hybridized to a probe derived from the origin-proximal end of the restriction fragment. This rendered visible the full-length restriction fragment and any smaller fragment(s). The size of that fragment(s) corresponded to the distance between the restriction site and the site(s) of inhibition.

Initially, the chromosomal DNA tested to locate the sites of inhibition was generated by replication of the chromosome from a temperature-inducible replication origin of a P2*sig5* prophage that was integrated near the terminus region. This was done so that we could control the timing of initiation and, thus, the timing of the use of the sites of inhibition. Also, since the P2*sig5* origin was located asymmetrically with respect to the terminus, forks should have entered the terminus from only one direction in the course of our experiments. The cells were first starved for required amino acids to prevent further initiation from *oriC* but allow ongoing replication to be completed. Replication from the prophage origin was induced by shifting the cells to 42°C. Initiation from *oriC* was prevented in these strains by the presence of a temperature-sensitive *dnaA* allele.

Figure 2 shows the results obtained after we probed for clockwise replication forks that were inhibited at T2. DNA was harvested at the indicated times after induction of replication from the P2*sig5* prophage intergrated at minute 16 (strain PK998). The restriction enzyme used was *Pst*I, which generated a 16.7-kb fragment that spanned from kb 429 to 446 between minutes 35 and 36 (Fig. 1). The probe that was used (pAP205) was a subcloned 2.2-kb fragment derived from the left end of that *Pst*I fragment at kb 429. The autoradiogram showed a termination band of 13.5 kb only after induction of replication from the prophage origin. The size of the termination band placed the site of inhibition at kb 442 on the physical map. This location was coincident with that of the T2 locus, as determined by the deletion experiments conducted by Hill et al. (10). That only one termination band was seen indicates that this is the only site of inhibition in this restriction fragment.

After the initial observation of the termination band, we found that we could identify the same band under nondenaturing conditions by taking advantage of the fact that the junction of a replication fork is expected to be excessively sensitive to shearing (15). Shearing should liberate the arm of a replication fork. Instead of denaturation, the DNA was sheared by vortexing it before it was digested with *Pst*I; and

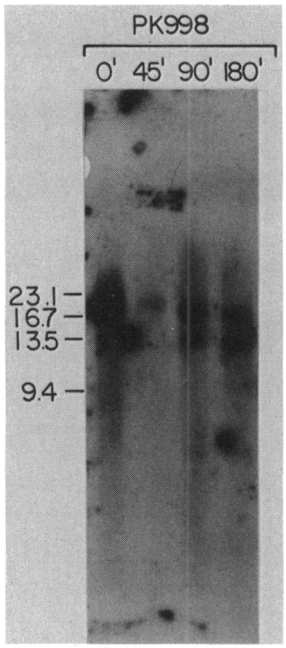

FIG. 2. Identification of the arrest site at T2. A Southern blot of an alkaline gel showing the appearance of a 13.5-kb band after induction of P2*sig5* in strain PK998. This strain had P2*sig5* integrated near minute 16, so that clockwise-traveling forks reached the terminus region before counterclockwise-traveling forks did. The probe (pAP205 in Fig. 1) was homologous to the left end of a 16.7-kb *Pst*I fragment from the T2 region. Samples were removed at the indicated times after induction of the prophage, and the positions of 23.1- and 9.4-kb size standards are indicated.

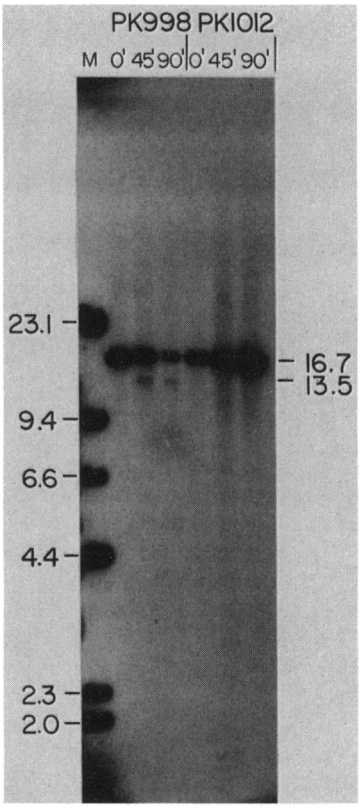

FIG. 3. Southern blot of a neutral gel showing the appearance of a 13.5-kb band after induction of P2*sig5*. The arm of the replication fork was released by shearing high-molecular-weight DNA followed by digestion with *Pst*I. pAP205 (Fig. 1) was used as the probe. Strain PK998 had the P2*sig5* prophage integrated near minute 16; clockwise-traveling replication forks reached the terminus first. Strain PK1012 had the P2*sig5* prophage integrated near minute 47; counterclockwise-traveling replication forks were the first to reach the terminus. Samples were removed at the indicated times after induction of the prophage origin. M indicates *Hin*dIII-digested λ DNA, with the sizes (in kilobases) indicated.

then it was fractionated on a nondenaturing gel, blotted, and probed as described above. Figure 3 shows that a band of the same size accumulated after induction of the P2*sig5* origin. Since this procedure was easier to perform and yielded identical results, we used it in subsequent experiments. It should be noted that when extremely gentle lysis and purification techniques were used, a slowly migrating band was detected (unpublished data). The nuclease sensitivity and other properties of this band indicate that it is analogous to band I described by Weiss and Wake (15), which was shown to be the intact Y structure of a replication fork.

The three lanes on the right of Fig. 3 show a control experiment in which T2 was replicated in the opposite direction from a P2*sig5* origin located at minute 47 on the opposite side of the terminus. In this strain, PK1012, no termination band was detected, demonstrating that the 13.5-kb band is not a nonspecific result of replication from the P2*sig5* origin and is dependent on the direction of replication. As an additional control confirming the known polarity of the site of arrest (5, 9), the right-hand side of the same *Pst*I fragment was probed in PK1012 (data not shown), and no termination band was detected.

We next wanted to identify where counterclockwise replication forks were inhibited at T1. Figure 4 shows results of a time course experiment after induction of replication from the P2*sig5* prophage at minute 47 (PK1012). The restriction enzyme used, *Hin*dIII, generated a 24-kb fragment spanning kb 75 to 99 near minute 28 (Fig. 1). The probe (pAP203) was a subcloned 1.4-kb fragment derived from the right end of the 24-kb *Hin*dIII fragment at kb 99. Once again, a single band appeared after induction of replication from the prophage origin. The size of the termination fragment indicates that T1 is at kb 90.

Hill et al. (10) have found a locus called *tus*, which is at or near T2 and encodes a *trans*-acting factor that is required for T1 to function. In strains containing a deletion of DNA from kb 434 to 444, it was demonstrated that the function of T1 was abolished, as judged by the marker-frequency assay. In order to demonstrate that our observed T1 band represented the same inhibition phenomenon that was seen in the marker-frequency assay, we tested such a strain for the function of T1. The middle two lanes in Fig. 4 show samples from 0 and 50 min after induction of replication from the P2*sig5* origin at minute 47 in the deletion strain PK2340, which was deleted for *tus*. The termination band could not be seen, even after long exposure. Consistent with the results of Hill et al. (10), the termination band was detected in the deletion strain if *tus* function was supplied from a plasmid (PK2374; Fig. 4). Strain PK1012 was included to show the location of the normal T1 band.

Since the termination bands appeared only after induction of replication from the asymmetrically placed P2*sig5* origins and since appearance of the termination band at T1 was dependent on the presence of *tus* function, we concluded

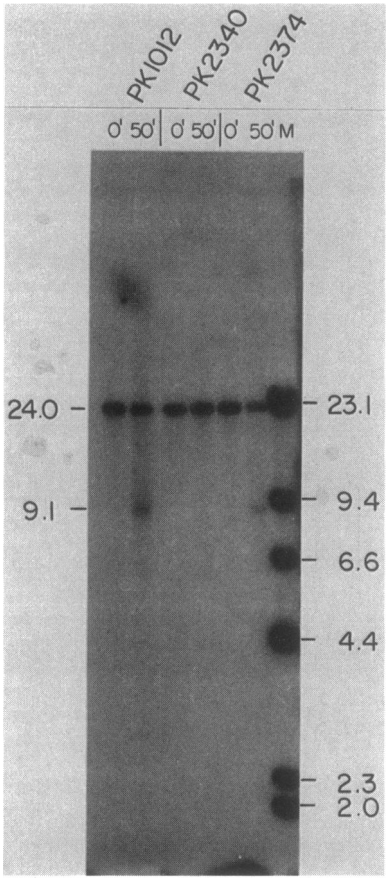

FIG. 4. Identification of the arrest site at T1 and dependence on *tus*. A neutral gel of sheared, *Hin*dIII-digested DNA was probed with pAP203 (Fig. 1), which is homologous to the right end of a 24-kb *Hin*dIII fragment from the T1 region. All strains shown had P2*sig5* integrated near minute 47; counterclockwise-traveling replication forks were the first to reach the terminus. PK1012 had a wild-type terminus region. PK2340 harbored a deletion of the *tus* locus. PK2374 had the same deletion but also contained a plasmid carrying the *tus* locus. M indicates *Hin*dIII-digested λ DNA, with the sizes (in kilobases) indicated.

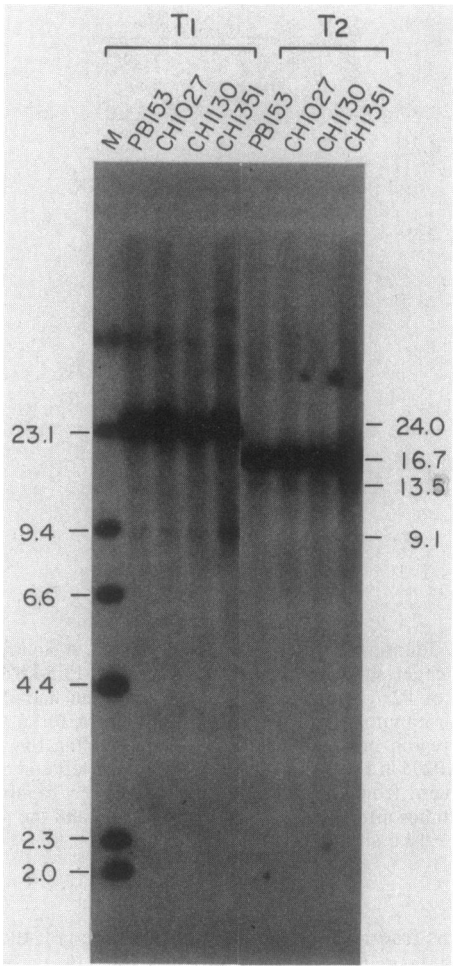

FIG. 5. Use of the sites of arrest during exponential growth. DNA harvested from cells growing exponentially in minimal medium was probed for arrested replication forks at both T1 and T2. pAP203 and pAP205 were used to visualize replication forks that were arrested at T1 and T2, respectively. Strain PB153 is an Hfr strain with a normal chromosomal arrangement. The other strains are derivatives of PB153 containing inversions between *rrnD* and *rrnE* (CH1130), *rrnD* and *rrnB* (CH1027), and *rrnG* and *rrnE* (CH1351). Sizes (in kilobases) are indicated.

that the bands represent inhibition of DNA replication at T1 and T2.

No inhibition was detected in adjacent upstream regions. Workers who have used various forms of marker-frequency assays have all reported a decrease in DNA replication upstream from the sites reported here (5, 9). In the experiments described above, it was noted that no other sites of arrest existed in the restriction fragments tested. In addition to testing those restriction fragments, we performed experiments to determine whether any other sites of inhibition were located farther upstream. Probes were chosen to locate sites of inhibition up to 50 kb upstream of T1 (between kb 90 and 140) and nearly 30 kb upstream of T2 (between kb 413 and 440). The restriction fragments chosen spanned most of the region in which a decline in marker frequency was observed. In each case, only the intact restriction fragment was detected (data not shown), indicating that no other sites of arrest exist in the region that was included in these restriction fragments. Further arguments concerning the existence of upstream sites are considered below.

The sites of arrest are used during exponential growth. It has been a question for some time whether T1 and T2 are used appreciably during growth in a normal cell, since previously, use of the sites could be detected only when the origin and terminus were positioned asymmetrically with respect to each other. In order to test this, DNA was harvested from exponentially growing cells of several strains. Strains were chosen to include a strain with a normal chromosomal arrangement as well as several strains containing inversions that produced various differences in the relative lengths of the two arms of the chromosome. The strains were otherwise isogenic. Figure 5 shows the results that were obtained for strain PB153, an Hfr strain which has the standard chromosomal arrangement, and the derivative inversion strains CH1130, which contains an inversion between *rrnD* (minute 72) and *rrnE* (minute 90); CH1027, which contains a comparable inversion between *rrnD* and *rrnB* (minute 90) (8); and CH1351, which contains a very large inversion between *rrnG* (minute 57) and *rrnE* (Charles Hill, personal communication).

The termination bands at both T1 and T2 were visible in all

of the strains. These bands were not detectable when DNA was harvested from stationary-phase cells or from cells that were starved for the required amino acids. In strains CH1130 and CH1027, the relative length of each arm of the chromosome was altered by about 15%. In strain CH1351, the clockwise distance to T2 was twice as long as the counterclockwise direction to T1; yet in all strains, use of both sites was detected. Also, when W3110, which contains an inversion between *rrnD* and *rrnE*, was compared with W1485, both sites were used (data not shown). This latter strain is the grandparent of W3110 and does not contain the inversion (8).

The inversion strain CH1351 reverts at a high frequency when cells are grown on rich medium (Charles Hill, personal communication). All the strains used in the comparison described above were grown in minimal medium to prevent reversion. Each of these inversion strains has a characteristic restriction pattern when they are digested with enzymes that have no sites in the *rrn* genes (Charles Hill, personal communication) (8). Accordingly, the DNA used in this experiment was tested for the presence of revertant DNA by Southern hybridization with cloned *rrnB* DNA (data not shown), and in each case it yielded only the pattern characteristic of the particular inversion. It should be mentioned that in other experiments in which other laboratory strains were grown in rich (LB) medium, the termination bands were more intense than those shown in Fig. 4.

DISCUSSION

We used a simple method for locating sites where inhibition of DNA replication occurs. This method was applied to the terminus of the *E. coli* chromosome, which previously has been shown to inhibit replication. We showed that inhibition occurs at a precise site of arrest at each edge of the terminus region and determined the location of both sites. These sites are in the regions defined by deletion analysis that contained the loci T1 and T2. Furthermore, we demonstrated that the sites of inhibition are used in exponentially growing cells.

The location of the arrest sites at kb 90 and 442 for T1 and T2, respectively, and the fact that they are actually single, precise sites was something of a surprise to us. We were aware that much of the upstream DNA is not essential for inhibition (10). However, marker-frequency determinations in strains containing asymmetrically placed P2*sig5* origins consistently demonstrated a decline in replication over a region of 50 kb or more, between kb 80 and 130 for T1 and kb 400 and 450 for T2. The likely explanation for this seemed to be that replication forks were being inhibited in this region. We speculated that there might be sites of inhibition distributed throughout the 50-kb region, to explain the apparent decrease in replication in that region.

Our experiments, which were designed to detect multiple sites of inhibition, gave a very different result. All detectable inhibition for each region occurred near the point that was lowest for new replication in the marker-frequency assay. We detected no replication forks that were arrested at specific sites in restriction fragments up to 50 kb upstream for T1 and 30 kb upstream for T2.

Furthermore, we know from the timing of the appearance of the termination band that the region upstream of each site of arrest had no strong effect on the progression of replication forks. There is about a 20 to 25-min lag period after the temperature shift before initiation of replication at the P2*sig5* origin (12). Each of the sites of arrest was located about 19 minutes from the P2*sig5* prophage that was used to detect it, and the first replication forks reached the terminus 40 to 45 min after the temperature shift (i.e., 20 to 25 min after initiation). This strongly suggests that at least the first replication forks to reach the terminus were not inhibited before they reached their arrest site.

The question still remains as to why a decrease in replication was detected consistently upstream of the actual sites of arrest when the marker-frequency assay was used on P2*sig5*-generated DNA. The simplest explanation is that the decreased replication was a result of prolonged P2*sig5* induction, which was necessary because of the low sensitivity of the marker-frequency assay. As noted above, the Southern hybridization assay used here detected the termination band at 45 min after induction, when only the first replication forks had reached the site of arrest. This is in contrast to results of the marker-frequency assay used in our laboratory, which required that the region adjacent to the site of arrest be amplified by being replicated several times in each cell for inhibition to be detected. Although we can exclude the possibility that the first forks to reach the terminus were inhibited strongly before they reached the indicated sites of arrest, subsequent forks may indeed have been slowed. It is possible that the effect of the later forks, which were backed up behind earlier forks, gives rise to the replication pattern seen in that system. This would not happen in a normal replication cycle. When DNA that was harvested at late times after induction of P2*sig5*, comparable to that used in the marker-frequency assay, was subjected to the Southern hybridization assay, no discrete sites of arrest were detected in the upstream region. However, broad smearing was occasionally seen in DNA that was harvested at these late times. This may reflect the same phenomenon that was detected in the marker-frequency assay.

From their marker-frequency data, de Massy et al. (5) have concluded that counterclockwise-traveling replication forks are slowed dramatically upstream of the sites of arrest that we identified, with this inhibition being strongest at kb 119 on the physical map. We checked this region for inhibition sites in our strains and detected none. Since we have not tested the strains used by de Massy et al. (5), we cannot exclude the possibility that some specific sites of arrest exist in their strains but not in ours. However, it is apparent from inspection of their data that the replication cycle of these strains was quite aberrant. It is possible that some of the effects seen in their experiments were the result of the perturbations to normal replication that existed in the system that they used.

Results of our experiment determined accurately the sites at which inhibition occurs. Results of additional experiments (data not shown) allowed us to locate each site within 100 base pairs. With some modifications, such as returning to denaturing gels and using polyacrylamide gel electrophoresis, resolution to the base pair level should be possible. By using strand-specific probes, we should also be able to detect any differences in inhibition between leading- and lagging-strand synthesis.

In order to determine more accurately those regions that are required for inhibition, we are applying this assay to strains that contain small deletions. Use of larger deletions coupled with the marker-frequency assay has already been instrumental in defining the region required for T2 function, which was determined to reside in a 4-kb region between kb 438 and 442, along with *tus*. Since this interval does in fact include the site of arrest, it now seems likely that this site and the locus required for inhibition are coincident or nearly

so. Accordingly, we have directed our deletion efforts toward the generation of small deletions near kb 442.

Deletions that include T1 have never been obtained, presumably because of the essential genes that are located near it. Strains that contain small deletions with only T1 removed should be viable, since we know that T1 function is not essential. Knowing exactly where the site of arrest is and assuming, as appears to be the case with T2, that the required locus is coincident with the site of arrest, we are generating small deletions at kb 90.

We determined that T1 and T2 are both used during exponential growth of normal cells, where replication is initiated from *oriC*. We cannot calculate accurately the frequency with which the sites are used without knowing some other factors. For example, the time required for resolution of the daughter chromosomes once the opposing replication forks reach T1 or T2 would influence how long the Y structure would persist, and thus how many could be detected at any given time. Experiments to resolve this are planned. At present, the simplest interpretation of the data is that one or the other site is used in many replication cycles.

Somewhat surprisingly, both inhibition sites were used in strains containing large changes in the relative lengths of each arm of the chromosome. Kohara et al. (11) have recently published a restriction map of the *E. coli* chromosome. From this map, we determined the physical distances between the origin and terminus in each strain used. In strains CH1027 and CH1130, the counterclockwise-replicated arm (terminating at T1) is shorter than the clockwise-replicated arm (terminating at T2) by about 350 kb. In strain PB153, the clockwise-replicated arm is shorter by about 130 kb. In these strains, which are otherwise isogenic, both sites are still used.

Even more extensive changes in the chromosome structure, in which the relative lengths of the arms were altered by a factor of 2, did not cause one site to be used exclusively. In the inversion strain CH1351, the clockwise distance to T2 is 3,470 kb, while the counterclockwise distance to T1 is only 1,690 kb. Use of T2 was still detectable in this strain, despite the fact that replication forks had to travel twice as far to reach it. It is possible that the cell compensates for the difference in distance by altering the replication rates so that the time required to replicate each arm is roughly the same. A similar effect has been postulated to occur in a *B. subtilis* strain in which the half-chromosomes differed considerably in length (7).

ACKNOWLEDGMENTS

We thank Jonathan P. Binkley, Roger Innes, Denise Lim, and Marianne Tecklenburg for helpful discussions through the course of this study and critical reading of the manuscript.

This study was supported by Public Health Service grant GM32968 from the National Institutes of Health (to P.L.K.) and a postdoctoral fellowship (to T.M.H.) from the National Institutes of Health.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Baran, N., A. Lapidot, and H. Manor.** 1987. Unusual sequence element found at the end of an amplicon. J. Mol. Cell. Biol. **7**: 2636–2640.
3. **Bastia, D., J. Germino, J. H. Crosa, and P. Hale.** 1981. Molecular cloning of the replication terminus of the plasmid R6K. Gene **14**:81–89.
4. **Bouché, J. P.** 1982. Physical map of a 470×10^3 base-pair region flanking the terminus of DNA replication in the *Escherichia coli* K12 genome. J. Mol. Biol. **154**:1–20.
5. **de Massy, B., S. Bejar, J. Louarn, J.-M. Louarn, and J.-P. Bouché.** 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.
6. **Gourse, R. L., Y. Takebe, R. A. Sharrock, and M. Nomura.** 1985. Feedback regulation of rRNA and tRNA synthesis and accumulation of free ribosomes after conditional expression of rRNA genes. Proc. Natl. Acad. Sci. USA **82**:1069–1073.
7. **Hanley, P. J. B., C. M. Carigan, D. B. Rowe, and R. G. Wake.** 1987. Breakdown and quantitation of the forked termination of replication intermediate of *Bacillus subtilis*. J. Mol. Biol. **196**: 721–727.

7a. **Hernandez, P., S. S. Lamm, C. A. Bjerknes, and J. Van'tHof.** 1988. Replication termini in the rDNA of synchronized pea root cells. EMBO J. **7**:303–308.

8. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:7069–7072.
9. **Hill, T. M., J. M. Henson, and P. L. Kuempel.** 1987. The terminus region of the *Escherichia coli* chromosome contains two separate loci that exhibit polar inhibition of replication. Proc. Natl. Acad. Sci. USA **84**:1754–1758.
10. **Hill, T. M., B. J. Kopp, and P. L Kuempel.** 1988. Termination of DNA replication in *Escherichia coli* requires a *trans*-acting factor. J. Bacteriol. **170**:662–668.
11. **Kohara, Y. K., A. Akiyamaas, 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.
12. **Kuempel, P. L., S. Duerr, and N. Seeley.** 1977. The terminus of the chromosome in *Escherichia coli* inhibits replication forks. Proc. Natl. Acad. Sci. USA **74**:3927–3931.
13. **Louarn, J., J. Patte, and J. M. Louarn.** 1977. Evidence for a fixed site of termination of chromosome replication in *Escherichia coli* K12. J. Mol. Biol. **115**:295–314.
14. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Weiss, A. S., and R. G. Wake.** 1984. A unique DNA intermediate associated with termination of chromosome replication in *Bacillus subtilus*. Cell **39**:683–689.
16. **Weiss, A. S., and R. G. Wake.** 1984. Impediment to replication fork movement in the terminus region of the *Bacillus subtilis* chromosome. J. Mol. Biol. **179**:745–750.