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Genetics

Terminus region of the chromosome in *Escherichia coli* inhibits replication forks

(bacteriophage P2*sig*$_5$/*rac* locus/DNA·DNA hybridization)

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ABSTRACT **Induction of prophage P2*sig*$_5$ at 42° caused replication of the bacterial chromosome in a *dnaA* mutant of *Escherichia coli*. The P2*sig*$_5$ is integrated in this strain near the *metG* locus, which is at min 47 on the genetic map. The regions of the chromosome replicated after prophage induction have been determined by means of DNA·DNA hybridization with various DNAs obtained from *Proteus mirabilis*/*E. coli* F′ merogenotes and from λ specialized transducing phage. The replication was initiated at the prophage site and was bidirectional. Most of the replication occurred in a counterclockwise direction on the genetic map, and the replication quickly proceeded to the *aroD* locus (min 37). The replication forks were retarded between *aroD* and *rac* (min 31) loci, although the *rac* locus was finally replicated. A more severe inhibition of replication occurred between the *rac* and *trp* (min 27) loci. It is proposed that the replication terminus is near the *rac* locus and that the terminus inhibits replication forks.**

In the processes involved in the termination of replication of the circular chromosome of *Escherichia coli*, it has been determined that the replication terminus is opposite the replication origin and is located somewhere between the *trp* and *his* loci, but the position of the replication terminus has not yet been reported with any greater precision (1–4). It is also not certain if the replication terminus is a definite locus on the chromosome or if it is simply wherever the two replication forks involved in bidirectional replication happen to meet. Various investigators have reported results that suggest that replication occurs in a unidirectional fashion in certain situations (5–7). This suggests that there might not be a locus between the *trp* and *his* loci that blocks replication forks.

Termination has now been studied with several different chromosomes that replicate bidirectionally, and the results demonstrate that bacteriophage λ (8) and simian virus 40 (9) do not possess a specific region of the chromosome that blocks replication forks. The plasmid R6K, however, does have a terminus that blocks replication forks (10). The *Bacillus subtilis* chromosome might also have a specific terminus (11–13).

In order to facilitate the study of termination in *E. coli*, we have sought conditions in which the normal symmetrical pattern of replication of the chromosome was altered so that one replication fork would reach the terminus region earlier than the other. We have recently determined that induction of prophage P2*sig*$_5$ causes bacterial chromosome replication from the site of insertion of the prophage, and one of the strains we have studied has the prophage integrated near the terminus region. The results of studies of this strain are reported here.

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MATERIALS AND METHODS

Bacterial Strains and Media. PK241 is an F$^-$ *thr leu his arg thi thyA drm dnaA malA*$^+$ *E. coli* strain derived from CRT4624. This latter strain and bacteriophage P2*tsD*$_4$*c*$_5$*sig*$_5$ (which will be called P2*sig*$_5$ in this paper) were obtained from Y. Hirota (14). PK289 is an F$^-$ *ilv arg met thi his thyA drm E. coli* strain. PM 14 is a *met trp his thr thy nic ilv Proteus mirabilis* strain. The PM14 F′ merogenote strains containing the F′129, F′116, F′111, F′101, and F′152 episomes were obtained by mating PM14 with *E. coli* strains containing these episomes and selecting for His$^+$, Thy$^+$, Ilv$^+$, Thr$^+$, and Gal$^+$ cells, respectively. The *E. coli* F′ strains were obtained from B. Bachman.

The sources of the λ specialized transducing phage were as follows: λgt-*E. coli Eco*RI *lop*-11 *lig*$^+$ 1 (λ*lig*), from R. Davis (15); λ*fla*N$^+$ 36 (λ*fla*), from M. Simon; λ reverse (λ*rev*), from M. Gellert (16); λ*ptrpED* 1 (λ*trp*), from C. Yanofsky (17); λ*c*I$_{am34}$*bio*256 (λ*bio*), from M. Furth. λ*aroD* was obtained by using the procedure of Schrenk and Weisberg (18).

Cells were usually grown in M9 medium (19). During incubation in the absence of required amino acids, arginine or methionine was not removed from the medium. When PK241-P2-1 cells were labeled with ^{32}P, they were grown in low-phosphate medium (20) containing 0.2 mM P$_i$ and ^{32}P-labeled orthophosphate, 0.5 mCi/ml.

DNA Isolation. The procedure of Marmur (21) was used to isolate DNA from the PM14 F′ merogenote strains. The strains were grown in 24-liter cultures to stationary phase in media that selected for the cells that maintained the episome. To isolate DNA from the λ specialized transducing phage, large volumes (4–24 liters) of high-titer lysates were concentrated by precipitation with polyethylene glycol (22) or by centrifugation at 18,000 × *g* for 4 hr. The phage were resuspended in a small volume of TM buffer (0.01 M Tris·HCl, pH 8.1/0.01 M $MgSO_4$) and incubated at 37° for 1 hr with DNase I, 10 μ/ml. The phage preparation was centrifuged twice at 110,000 × *g* for 14 hr in CsCl gradients (ρ = 1.5), and the phage were then dialyzed against TM at 5°. The preparation was incubated again with DNase I and extracted four times with phenol, and the DNA was then dialyzed against 0.15 M NaCl/0.015 M sodium citrate, pH 7 (21).

DNA·DNA Hybridization Procedures. The procedures used to attach purified DNA to Millipore filters, to dry and preincubate the filters before hybridization, and to wash them after hybridization have been described (20). For the hybridizations with the merogenote DNAs, 5 μg of DNA was attached to the

Abbreviation: P2*sig*$_5$, bacteriophage P2*tsD*$_4$*c*$_5$*sig*$_5$.

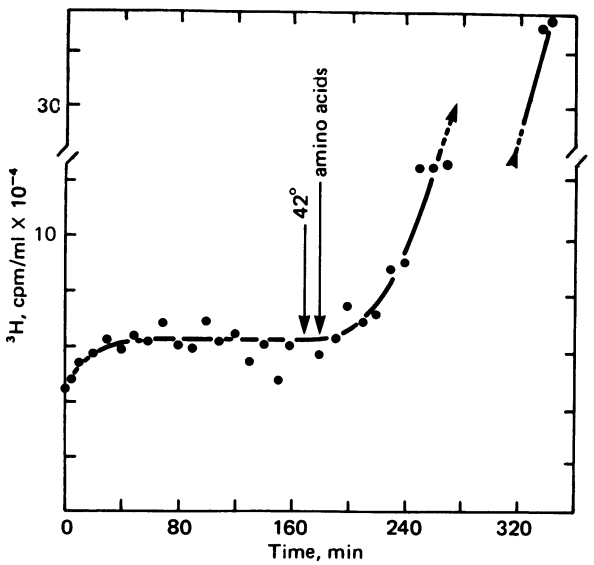

FIG. 1. DNA synthesis in PK241-P2-1 cells during incubation in the absence of required amino acids and after induction of P2*sig*$_5$ at 42° in complete medium. Cells were grown exponentially for many generations at 28° in medium containing [^{3}H]thymine (0.31 Ci/mmol) and then transferred to radioactive medium lacking the required amino acids. The time of this transfer is shown as 0 min. The subsequent increase in trichloroacetic acid-precipitable radioactivity was then determined. The cells were incubated for 170 min, transferred to 42°, and the required amino acids were added to the medium at 180 min.

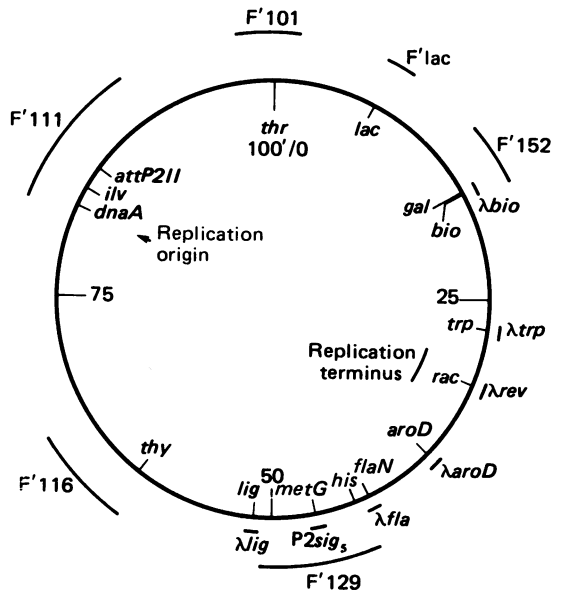

FIG. 2. Genetic map of *E. coli*, showing the locations of pertinent genes (23), the regions contained in the PM14 F′ merogenote DNAs used for DNA·DNA hybridizations (24), and the loci present in the λ specialized transducing phage used in this study (in minutes).

filters, the filters were preincubated for 6 hr at 65° with 1 ml of preincubation medium, and 0.1 ml of solution was then added that contained 20 μg of *P. mirabilis* DNA, 0.18 μg of [^{14}C]DNA, and 0.02 μg of [^{3}H]DNA. The [^{14}C]DNA was obtained from exponentially growing PK289 cells that had grown for many generations in medium containing [^{14}C]thymine (56 mCi/mmol) and were incubated an additional 150 min in medium containing [^{14}C]thymine but lacking the required amino acids. The [^{3}H]DNA was obtained from PK241-P2-1 cells in which the prophage had been induced. The DNAs used in the hybridization had been hydrolyzed in alkaline solution to decrease their molecular weights (20). The hybridizations were incubated at 65° for 18 hr.

The hybridizations with DNA obtained from λ specialized transducing phage were conducted with 1 μg of DNA attached to the Millipore filters. The filters were preincubated for 6 hr at 37° in preincubation medium containing 45% formamide. The hybridizations were conducted with 1 ml of preincubation medium that contained 45% formamide, 20 μg of λ DNA, 0.3 μg of [^{32}P]DNA, and 0.2 μg of [^{3}H]DNA. Prior to incubation with the filters, the hybridization solutions were incubated at 37° for 6 hr to preanneal any sequences in the radioactive DNAs that were homologous to λ DNA. The preannealed hybridization solutions and the filters were then incubated together for 18 hr at 37°. The DNAs in the hybridization solutions had been hydrolyzed in alkaline solutions to reduce their molecular weights. The [^{32}P]DNA was purified from PK241-P2-1 cells that had been grown to stationary phase in limiting phosphate medium containing ^{32}P-labeled orthophosphate (0.5 mCi/ml) and 0.2 mM P_i. All hybridizations were conducted in triplicate.

RESULTS

The bacterial strain used in these studies was derived from PK241, a *dnaA* mutant that is temperature sensitive for the initiation of chromosome replication. PK241 was lysogenized with bacteriophage P2*sig*$_5$. This phage has a temperature-sensitive repressor, is blocked in the formation of phage tails at 42°, and presumably contains a small insertion between the *C* and *B* genes (14). Approximately one-half of the lysogens that we tested grew at 42°. These lysogens were assumed to be integratively suppressed by insertion of the prophage at the *attP2II* site (14), and they were not studied further. Strain PK241-P2-1, which is the subject of this paper, did not grow at 42°. We have mapped the site of insertion of P2*sig*$_5$ by means of conjugation and transduction. The prophage is at a site that is at approximately min 47 on the genetic map, because it is 70% cotransducible with *metG* (unpublished data).

When PK241-P2-1 cells were incubated at 42°, DNA synthesis occurred due to the induction of the prophage. In the experiment shown in Fig. 1, exponentially growing cells were incubated for 170 min at 28° in the absence of required amino acids in order to cause completion of existing replication cycles. The cells were then shifted to 42°, and the required amino acids were added. DNA synthesis resumed after a lag of 30 min, and the total DNA increased approximately 6-fold in 3 hr. No increase in DNA at 42° was observed when a comparable experiment was conducted with the nonlysogenic strain PK241.

To determine the regions of the bacterial chromosome that were replicated after induction of the prophage, we isolated the DNA from a number of different *P. mirabilis* F′ merogenotes (Fig. 2). The DNA·DNA hybridization procedure utilizing the merogenote DNAs was similar to that described by Yahara (26). The major difference was that we did not separate the *E. coli* DNA from the *P. mirabilis* DNA. Instead, we added a large excess (18 μg/ml) of nonradioactive *P. mirabilis* DNA to the hybridization solutions. This reduced the high backgrounds that otherwise resulted from nonspecific hybridization of radioactive *E. coli* DNA to the *P. marabilis* DNA present in the merogenote DNA bound to the nitrocellulose filter. The 1.1 ml of hybridization solution also contained 0.02 μg of DNA from PK241-P2-1 cells that were labeled with [^{3}H]thymine after induction of the P2*sig*$_5$ prophage and 0.18 μg of [^{14}C]DNA from cells that

Table 1. Hybridizations of induced DNA with PM14 F′ merogenote DNAs

	Merogenote DNAs						
	PM14	F′129	F′116	F′111	F′101	F′*lac*	F′152
				At 55 min			
^{3}H cpm/^{14}C cpm	17/77	476/237	37/286	7/200	12/151	43/250	35/169
Ratio	—	2.01	0.13	0.04	0.08	0.17	0.21
Normalized ratio	—	2.01	0.13	0.04	0.08	0.17	0.21
				At 80 min			
^{3}H cpm/^{14}C cpm	47/72	1019/262	180/312	11/176	29/148	84/254	95/194
Ratio	—	3.89	0.58	0.06	0.20	0.33	0.49
Normalized ratio	—	5.02	0.75	0.08	0.26	0.43	0.63
				At 120 min			
^{3}H cpm/^{14}C cpm	105/75	1576/250	468/318	33/144	57/141	113/204	170/191
Ratio	—	6.30	1.47	0.23	0.40	0.55	0.89
Normalized ratio	—	11.40	2.66	0.42	0.72	1.00	1.61
				At 160 min			
^{3}H cpm/^{14}C cpm	216/74	2256/238	1223/329	239/165	187/154	266/207	304/173
Ratio	—	9.48	3.72	1.45	1.21	1.29	1.76
Normalized ratio	—	14.13	5.54	2.16	1.80	1.92	2.62

PK241-P2-1 cells were incubated in the absence of required amino acids for 170 min at 28° and for 10 min at 42°; then the amino acids and [^{3}H]thymine (5.5 Ci/mmol) were added. Samples removed at the indicated times were hybridized with PM14 F′ merogenote DNAs attached to nitrocellulose filters. The hybridization to PM14 DNA is background. The data in the other columns have been corrected for this background. The input radioactivities for the hybridizations were as follows: at 55 min, 2351 cpm of ^{3}H and 3660 cpm of ^{14}C; at 80 min, 4035 cpm of ^{3}H and 3743 cpm of ^{14}C; at 120 min, 6832 cpm of ^{3}H and 3794 cpm of ^{14}C; at 160 min, 11,681 cpm of ^{3}H and 4003 cpm of ^{14}C. The normalized ratio corrects for the different amounts of the induced culture that were used to prepare the 55, 80, 120, and 160 min hybridization solutions.

had been incubated in the absence of required amino acids and consequently had all genes present at equal frequency (1).

The hybridizations were conducted with 5 μg of *P. mirabilis* F′ merogenote DNA attached to the nitrocellulose filters. The data are presented in the form of [^{3}H]DNA cpm/[^{14}C]DNA cpm that hybridized to the various merogenote DNAs. This ratio was calculated after subtracting the background hybridization that occurred on filters containing 5 μg of *P. mirabilis* DNA. The use of the ratio corrects for the different sizes of the episomes; more [^{3}H]DNA and [^{14}C]DNA will hybridize to filters containing DNA obtained from the larger episomes, other things being equal. Differences in this ratio will reflect the amount of [^{3}H]DNA, relative to the normalizing amount of [^{14}C]DNA, that comes from the region of the chromosome contained in the episome.

In an experiment to determine the regions of the bacterial chromosome replicated after induction of the prophage, cells previously deprived of required amino acids were shifted to 42°, and the amino acids and [^{3}H]thymine were added. Samples to be used for DNA·DNA hybridization were removed 55, 80, 120, and 160 min later. The amount of DNA increased after induction and, because the hybridizations were conducted with equivalent amounts of DNA, decreasing amounts of the induced culture were used to make the 55, 80, 120, and 160 min hybridization solutions. Table 1 shows the [^{3}H]DNA cpm/[^{14}C]DNA cpm ratios obtained with these solutions for DNA·DNA hybridization, as well as the normalized ratios (corrected for the reduced amounts of culture used to prepare the 80, 120, and 160 min hybridization solutions).

The normalized ratios are plotted in Fig. 3, and the pattern of replication was completely different from that usually observed in *E. coli*. The replication presumably started at the prophage site, and it proceeded clockwise around the chromosome, towards the replication origin. The normal replication origin was not used in these conditions. The amount of replication in the region of the *gal* locus (min 17) indicates that replication also occurred counterclockwise from the prophage. The merogenote DNAs did not allow an accurate determination of the amount of replication that occurred in the counterclockwise direction near the replication terminus.

We isolated DNA from various λ specialized transducing phage in order to study the replication that occurred in the immediate vicinity of the replication terminus. Fig. 2 shows these phage and the regions of the *E. coli* chromosome that have been inserted in them. The figure shows that λ*rev* has incorporated the *rac* locus. A number of genetic observations indicate this (16), and we tested this directly by means of DNA·DNA hybridization. The DNA inserted in λ*rev* is present on the F′123 episome, which contains the region from *trp* through *rac*. We have also determined that strain AB1157 (Rac$^-$) is missing most or all of the DNA sequences that are inserted in λ*rev* (N. Seeley and P. Kuempel, unpublished data).

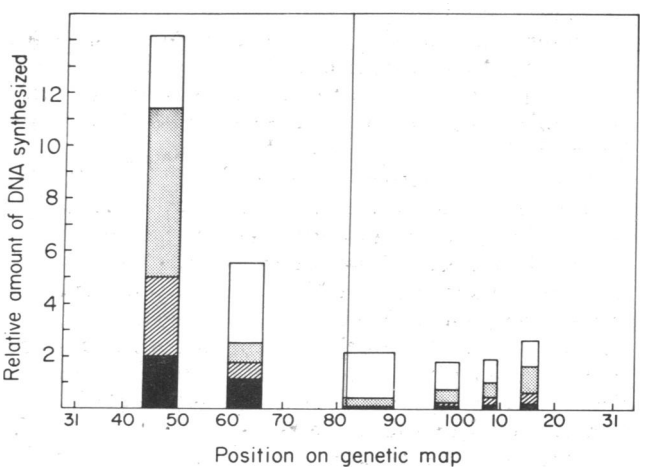

FIG. 3. Relative amounts of replication in different regions of the *E. coli* chromosome after induction of P2*sig*$_5$ in PK241-P2-1. The ordinate presents the normalized ratios from Table 1. The abscissa shows the positions on the *E. coli* genetic map of the episome DNAs used for hybridization. The circular genetic map is broken at the *rac* locus (min 31), which is at or near the replication terminus. ■, 55 min; ▨, 80 min; ▩, 120 min; and □, 160 min.

Table 2. Hybridizations of induced DNA with λ specialized transducing phage DNAs

	Phage DNA							
	λ+	λ*lig*	P2*sig*$_5$	λ*fla*	λ*aroD*	λ*rev*	λ*trp*	λ*bio*
				At 55 min				
^{3}H cpm/^{32}P cpm	257/301	309/890	7682/3024	2226/950	1696/746	1232/1136	113/310	11/517
Ratio	—	0.35	2.54	2.34	2.27	1.08	0.36	0.02
Normalized ratio	—	0.35	2.54	2.34	2.27	1.08	0.36	0.02
				At 80 min				
^{3}H cpm/^{32}P cpm	215/208	331/863	5334/2973	1804/1009	1265/699	1149/1118	94/375	0/560
Ratio	—	0.38	1.79	1.79	1.81	1.03	0.25	0.00
Normalized ratio	—	0.75	3.54	3.54	3.58	2.04	0.50	0.00
				At 120 min				
^{3}H cpm/^{32}P cpm	536/290	1026/916	8013/3016	2467/982	2087/705	2181/994	190/420	0/430
Ratio	—	1.12	2.66	2.51	2.96	2.19	0.45	0.00
Normalized ratio	—	1.71	4.07	3.84	4.53	3.35	0.69	0.00
				At 160 min				
^{3}H cpm/^{32}P cpm	384/203	1562/978	8730/3157	2729/1057	2299/743	2806/1142	411/450	121/523
Ratio	—	1.60	2.77	2.58	3.09	2.46	0.91	0.23
Normalized ratio	—	3.33	5.76	5.37	6.43	5.12	1.89	0.48

PK241-P2-1 cells were induced as described in the legend for Table 1, except that the specific activity of the [^{3}H]thymine was 12.4 Ci/mmol. Samples removed at the indicated times were hybridized with the various phage DNAs attached to nitrocellulose filters. The hybridization to λ DNA is background. The data in the other columns have been corrected for this background. The input radioactivities for the hybridizations were as follows: at 55 min, 235,700 cpm of ^{3}H and 555,000 cpm of ^{32}P; at 80 min, 223,800 cpm of ^{3}H and 612,100 cpm of ^{32}P; at 120 min, 491,000 cpm of ^{3}H and 663,600 cpm of ^{32}P; at 180 min, 505,900 cpm of ^{3}H and 597,100 cpm of ^{32}P. The normalized ratio corrects for the different amounts of the induced culture that were used to prepare the 55, 80, 120, and 160 min hybridization solutions.

The rationale of the hybridizations with the phage DNAs was similar to that previously described for the merogenote DNAs. The 1 ml of solution used for the individual hybridizations contained 20 μg of λ wild-type DNA, 0.2 μg of [^{3}H]DNA from induced cells, and 0.3 μg of [^{32}P]DNA. The [^{32}P]DNA was obtained from PK241-P2-1 cells grown to stationary phase on limiting amounts of ^{32}P, and this DNA was assumed to contain all genes at equal frequency. The λ wild-type DNA was included to reduce the backgrounds in the hybridizations. Table 2 presents the data obtained from hybridizations conducted with samples removed at 55, 80, 120, and 160 min after prophage induction. Filters containing 1 μg of P2 DNA were also included in these hybridizations to determine the amount of P2*sig*$_5$ replication that occurred. The normalized ratios, plotted in Fig. 4, show that replication was initiated at P2*sig*$_5$ and that most of the replication occurred initially in a counterclockwise direction towards the replication terminus. At later times, however, approximately one-half of the replication forks traveled in a clockwise direction towards the region of the replication origin. At all of the times examined, comparable amounts of replication were observed for P2*sig*$_5$ and the *fla* and *aroD* loci. In the later samples, the amount of replication at the *rac* locus finally equalled that observed for P2*sig*$_5$, *fla*, and *aroD*. The amount of replication at the *trp* and *bio* loci never equalled that attained at these other loci.

DISCUSSION

The experiments reported here demonstrate that induction of prophage P2*sig*$_5$ in strain PK241-P2-1 caused replication of the bacterial chromosome. This replication was initiated at the site of integration of the prophage, which is near the *metG* locus at min 47 on the genetic map. The replication of the chromosome proceeded in both directions (Figs. 3 and 4), but the replication was primarily unidirectional at early times after induction of the prophage (Fig. 4). Consequently, the bidirectional replication we have observed is not inconsistent with the observations of Schnöss and Inman (27) that replication of the P2 chromosome is unidirectional. In addition, if the P2*sig*$_5$ integrated near *metG* in PK241-P2-1 has the same orientation as P2 integrated near *metG* in *E. coli* C strains (25), the known direction of replication of the P2 chromosome (27) predicts that the replication of the bacterial chromosome should have proceeded in a counterclockwise direction. We do not know why some of the replication also proceeded in the clockwise direction, but reduced replication in one direction also occurred when P2*sig*$_5$, integrated at other sites in the chromosome, was induced (unpublished data).

The replication that occurred in the counterclockwise direction quickly proceeded as far as the *aroD* locus (Fig. 4). Prophage P2*sig*$_5$, and the *fla* and *aroD* loci all were replicated to the same extent, and there was no indication of a gradient of replication in this region. This suggests that the replication forks traveled rapidly in this region, relative to the frequency

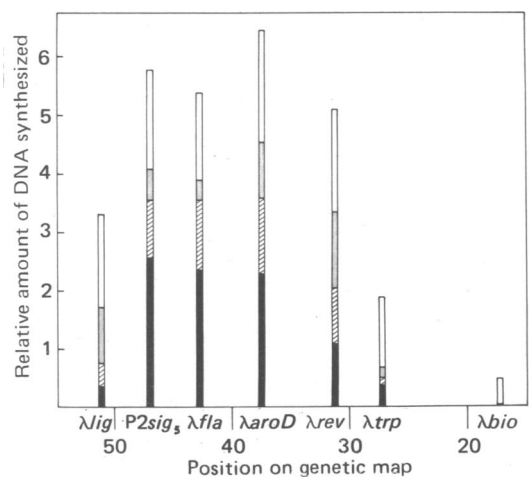

FIG. 4. Relative amounts of replication near the replication terminus after induction of P2*sig*$_5$ in PK241-P2-1. The ordinate presents the normalized ratios from Table 2. The abscissa shows the positions on the *E. coli* genetic map of the integration site of P2*sig*$_5$ and the regions present in the various λ specialized transducing phage. ■, 55 min; ▨, 80 min; ▩, 120 min; and □, 160 min.

of initiation at P2*sig*$_5$. Experiments utilizing autoradiographic procedures have demonstrated that the replication forks traveled at a minimum rate of 20 μm/min (unpublished data).

The replication in the counterclockwise direction is impeded somewhere between the *aroD* and *rac* loci. Although *rac* is separated from *aroD* by a maximum of 82 μm of DNA, replication of *rac* occurred 25–40 min after replication of *aroD*. For example, the amount of replication at *rac* was much less than at *aroD* at 80 min (Fig. 4), but at 120 min the amount of replication at *rac* equaled that at *aroD* at 80 min. An even longer lag, approximately 80 min, occurred between the replication of the *rac* and *trp* loci, even though these loci are separated by a maximum of 55 μm of DNA.

The region that impedes the passage of the replication forks corresponds to the large region on the *E. coli* genetic map that is near the replication terminus and that contains very few known loci (23). The genetic map distances in this part of the chromosome have only been determined by time-of-entry data obtained from bacterial conjugation. It is possible that this region also impedes the transfer of the chromosome during conjugation and that it does not contain the usual 13.7 μm of DNA per min of the genetic map. If this region does not contain much DNA, the *aroD*, *rac*, and *trp* loci are physically quite close to each other on the chromosome, their positions being comparable to those shown in the previous genetic map of *E. coli* (28). If this is so, the inhibition of replication at the terminus is even more dramatic than it appears in Fig. 4, in which the abscissa shows the relative positions of these loci according to the most recent genetic map of *E. coli* (23).

The replication beyond the *aroD* locus is not impeded merely because the replication forks initiated by P2*sig*$_5$ can only replicate a short distance on the chromosome. Fig. 3 shows that the replication in the clockwise direction proceeded at least as far as the replication origin, which is separated from the prophage by approximately 480 μm of DNA. The actual gradient of replication in this direction is not as steep as it appears. The P2*sig*$_5$ is integrated at a site near min 47 on the chromosome, which corresponds to a position close to the middle of the DNA contained in the F′129 episome (Fig. 1). The [^{3}H]DNA that hybridized to this episome consequently arose from replication forks that started at P2*sig*$_5$ and traveled in opposite directions. Because replication forks that traveled in the clockwise direction at 160 min were only one-half as frequent as replication forks that traveled in the counterclockwise direction (Fig. 4), approximately one-third of the [^{3}H]DNA from the 160-min sample that hybridized to the F′129 episome DNA came from replication forks that traveled in the clockwise direction. If it had been possible to conduct the hybridizations with an episome that only contained the region between min 47 and 50, the [^{3}H]DNA cpm/[^{14}C]DNA cpm ratio would have been approximately two-thirds of that shown in the graph. Conversely, the replication in the counterclockwise direction is probably underestimated about 1.33-fold in the hybridizations with the F′129 episome.

Analyses of strains in which P2*sig*$_5$ caused integrative suppression of *dnaA* mutations have also demonstrated that P2*sig*$_5$ can replicate long regions of the chromosome. The P2*sig*$_5$ is integrated at the *attP2II* site in these strains (Fig. 2), and the bidirectional replication that occurred following induction of the prophage proceeded all the way to the replication terminus (P. Maglothin and P. Kuempel, unpublished data).

The nature of the region at the terminus of the chromosome that impedes the passage of replication forks is completely obscure. The information that this region exists, however, and its location should lead to techniques that will examine this part of the chromosome more closely. The *rac* locus and λ*rev* are of obvious use in such studies, because they supply the only available fragment of DNA from this part of the chromosome. We are presently studying chromosome replication in another P2*sig*$_5$ lysogen, in which the prophage is integrated near the *gal* locus. Preliminary experiments indicate that induction of the prophage caused initiation of replication that rapidly proceeded in a clockwise direction from the *gal* locus as far as the *rac* locus, and the replication forks were then sharply inhibited (unpublished data).

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