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Suppression of an *Escherichia coli dnaA* Mutation by the Integrated R Factor R.100.1: Change of Chromosome Replication Origin in Synchronized Cultures

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We have followed, by deoxyribonucleic acid-deoxyribonucleic acid hybridization, the order of replication of three chromosomal markers during a synchronous round of replication in three strains of *Escherichia coli* carrying a $dnaA_{ts}$ mutation: one strain in which the F-like R factor R.100.1 was established as a plasmid and two strains in which the *dnaA* mutation was suppressed by the integration of R.100.1 into the chromosome. In the R^+ strain at 30 C, replication of the plasmid took place simultaneously with the initiation of chromosome replication at the normal origin. In the integratively suppressed Hfr strains, at 42.5 C, chromosome replication was initiated preferentially from the integrated plasmid; little or no initiation occurred at the normal origin. Similar results were obtained for the one strain tested at 30 C. For both Hfr strains at 42.5 C, the data suggest that at least part of the population replicated bidirectionally. This conclusion has been confirmed using an autoradiographic procedure. Both types of experiment indicate a wide variation in the rate of travel of individual replication forks within the population.

The $dnaA_{ts}$ mutants of *Escherichia coli* seem to be impaired in the initiation of chromosome replication at temperatures above 40 C (7, 24). Under certain conditions, the effect of the *dnaA* mutation can be suppressed by the integration of a plasmid into the host chromosome. This suppression may be mediated by any one of several different plasmids, and integration may occur at any one of many chromosomal sites (15, 16).

To explain this phenomenon of "integrative suppression," we proposed a model according to which the initiation of chromosome replication takes place at the initiation site of the integrated plasmid, the chromosome thus becoming part of the plasmid replicon (15). The evidence for this interpretation was indirect: it was shown that the suppressed strain still carries the *dnaA* mutation, that integration of the plasmid is essential, and that loss of the integrated plasmid results in a reversion to a temperature-sensitive phenotype. In strains suppressed by the integration of an F factor, it was shown that chromosome replication is sensitive to acridine orange at 41 C but not at 30 C; this implies that at the nonpermissive temperature replication is under the control of the F factor (15). Further support for the model has been the demonstration that, during integrative suppression by the prophage P2*sig*, chromosome replication is dependent on the A function of P2 (12). Thus, whereas the available evidence was consistent with our original hypothesis, no direct demonstration of a change in the origin of replication, during integrative suppression, had been obtained.

To approach this question in a more direct fashion, we have followed, by deoxyribonucleic acid (DNA)-DNA hybridization, the order of replication of three chromosomal markers, the integrated plasmid R.100.1 and the prophages λind^- and Mu::*ilv* (i.e., Mu-1 integrated into *ilv*), during a synchronous round of replication in two *dnaA* strains suppressed by the integration of the F-like R factor R.100.1. It has previously been shown that the integration of this plasmid gives rise to stable Hfr's with transfer properties similar to those of F-derived Hfr's (16). We have also examined the replication of newly initiated chromosomes by the autoradiographic procedure of Prescott and Kuempel (19).

We conclude that: (i) in synchronized cultures of the parental R^+ strain, MUT26 ($dnaA_{ts}$/R.100.1), growing at 30 C, the first round of replication is initiated simultaneously on both R.100.1 and the chromosome; (ii) in synchro-

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nized cultures of two Hfr strains growing at 42.5 C, initiation occurs predominantly within the integrated R factor; (iii) in synchronized cultures of one Hfr strain, analyzed at 30 C, replication from the integrated R factor is the predominant mode; (iv) replication of at least a fraction of the Hfr chromosomes at 42.5 C proceeds in a bidirectional manner; (v) the replication fork velocity for the Hfr's studied differs from that of the parent at both 30 and 42.5 C.

MATERIALS AND METHODS

Bacteria and bacteriophages. The bacterial strains used in this study are given in Table 1. The Hfr derivatives of strain MUT26 were isolated and characterized by previously published techniques (16). The relative chromosome positions of the integrated plasmid and of the prophages in these strains is shown in Fig. 1.

Bacteriophages Mu-1 and λcI857S7 were from our collection. Growth of Mu-1 has been described (2). Lambda stocks were prepared by thermal induction of bacteria lysogenic for λcI857S7.

Strains LC468 (*leu thy*/R.100.1) and LC524 [*leu thy* (*proB lac*)$_{del}$/RTF-TC], both derivatives of *E. coli* W1485, were used in the preparation of R.100.1 and RTF-TC DNA, respectively. The RTF-TC is a plasmid derived from R.100.1; it has a molecular weight

TABLE 1. *Bacterial strains*

Strain	Sex	Description
CB0129	F⁻	*thi leu thy*: a derivative of W1485 (reference 4)
LC148	F⁻	(*proB lac*)$_{del}$ in CB0129
LC366	F⁻	*ilv* derivative of LC148 by nitrosoguanidine mutagenesis (from J. M. Louarn)
LC343	F⁻	LC366 *dnaA*46: made by cotransduction with *ilv*⁺ from CRT46
LC468	R⁺	CB0129 carrying R.100.1[a]
LC524	R⁺	LC148 carrying RTF-TC[b]
CRT46	F⁻	*thi thr leu thy lac mal* (Hirota et al., 1968 [7])
MUT26	R⁺	LC343 *ilv*: Mu-1, λ*ind*⁻ carrying R.100.1[c]
LC2602	Hfr	Derivative of MUT26 able to grow at 42 C; transfer 0-*proA proB* .... *ara* R
LC2633	Hfr	Derivative of MUT26 able to grow at 42 C; transfer 0-*serA lys* .... *argG* R

[a] Egawa and Hirota (6). This R factor carries resistance to streptomycin, chloramphenicol, sulphanilamide, and tetracycline.

[b] A derivative of the R factor, R.100.1, lacking resistance to streptomycin, chloramphenicol, and sulphonilamide. Isolated by A. Rifat.

[c] Mu-1 was integrated into *ilv* by the technique of Taylor (22).

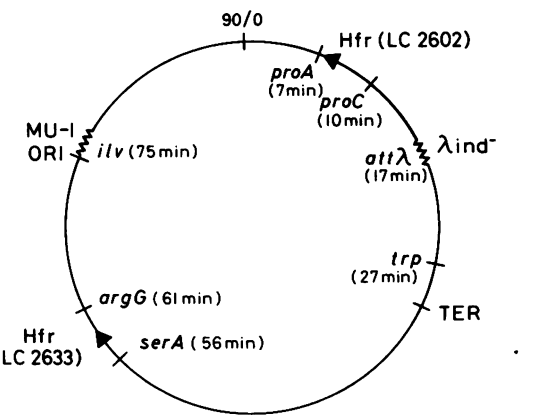

FIG. 1. *Genetic map of E. coli K-12 showing the points of origin and the direction of chromosome transfer in the two Hfr's used. The map positions shown are from the standard E. coli genetic map (23).*

80% that of R.100.1, and it lacks all the drug resistance markers except tetracycline resistance but has retained the transfer properties of R.100.1 (17). The RTF-TC-carrying strain that we used was isolated and characterized by A. Rifat and R. Epstein (personal communication).

Growth media. The strains were grown in glucose M9 medium (1) supplemented with 10 μg of thymine per ml and 0.4% Casamino Acids (Difco). Strains used for preparation of phage and plasmid DNA were grown in L-broth (11). Medium changes were made by collecting cells on a Sartorius membrane filter, washing them with prewarmed M9 medium, and suspending them in the desired medium at the required temperature. Cell growth was monitored by adsorption at 450 nm (A_{450}) in an Hitachi spectrophotometer.

Pulse labeling. Cultures for pulse labeling were pipetted or poured rapidly into prewarmed tubes containing radioactive thymidine (New England Nuclear Corp.) at a final concentration of 0.5 μg/ml (final specific activity, 20 Ci/mmol). The pulse was terminated after 2 min by pouring the cultures onto crushed, frozen medium containing 5 to 10% pyridine. The cells were collected by centrifugation, washed with 0.02 M tris(hydroxymethyl)aminomethane, 0.05 M ethylenediaminetetraacetate (pH 8), and stored at −20 C.

DNA extraction. The extraction and purification of bacteriophage DNA and that of pulse-labeled *E. coli* DNA have been described previously (2, 13). Plasmid DNA from strains LC468 and LC524 was isolated from stationary-phase cultures grown in L-broth, in the form of covalently closed circles, according to Clewell and Helinski (5).

Hybridization procedure. Hybridization was carried out on Sartorius membrane filters (MF50). The method of preparation and treatment of filters prior to hybridization was that of Louarn et al. (13), except that plasmid DNA was boiled for 10 to 15 min in 0.1 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium

citrate) before applying to filters. Electron microscopy indicated that this procedure was sufficient to disrupt more than 90% of the covalently linked circular molecules.

A 0.5-ml volume of the sonicated denatured bacterial DNA, containing 5,000 to 10,000 counts/min, in 2× SSC and 50% formamide was added to each filter (8). Samples of the labeled bacterial DNA were hybridized to filters supporting 2 μg of *E. coli* (from CB0129), λ, Mu-1, R.100.1, or RTF-TC DNA. The reaction was carried out at 42 C for 5 days. Filters were washed and counted in a Packard Tri-Carb liquid scintillation counter (13). Under these conditions, hybridization of all the DNA species except for bulk *E. coli* DNA reaches saturation well within this time (data not shown).

The hybridized counts are expressed as a percentage of the counts binding to filters supporting *E. coli* DNA. This treatment corrects for the differences in hybridization efficiency from sample to sample. The values obtained depend, therefore, on the extent of the *E. coli-E. coli* hybridization reaction and may vary from experiment to experiment. The data were not corrected for the different molecular weights of the three DNA species used nor for their different hybridization efficiencies. It is clear, therefore, that although the shape of the curve for a marker reflects accurately the course in time of its replication, quantitative comparisons between markers are not meaningful. To estimate the degree of nonspecific binding of bulk *E. coli* DNA to the various DNA species bound to the filters, pulse-labeled DNA from an exponentially growing culture of strain CB0129 (R^-, λ^-, Mu-1^-) was hybridized against each of the DNA species in each experiment. The counts hybridized were also expressed as a percentage of the counts binding to the control *E. coli* DNA and subtracted from the experimental values.

Pulse labeling of synchronized cultures. Starvation of a bacterial culture for a required amino acid (14), followed by a period of thymine starvation in the presence of amino acids (20), results in the synchronous initiation of a round of replication upon readdition of thymine (13).

To synchronize replication, cells were grown in M9 glucose medium, supplemented with 10 μg of thymine per ml and 10 μg of Casamino Acids per ml, to an A_{450} of about 0.3; they were starved for amino acids for 2 h in the presence of thymine and then starved for thymine in the presence of amino acids for 1 h (a time sufficient for the culture mass to double). Replication was reinitiated by the addition of 10 μg of thymine per ml. At various times after this step 2-ml samples of the culture were pulse labeled with [^{3}H]thymidine. The labeled DNA was extracted, purified, and hybridized to the series of test DNAs.

The number of free Mu-1 or λ phage in the cultures, after release from thymine starvation, was always less than 1 in 10^3 bacteria and thus does not significantly affect the results.

Autoradiography. The method was adapted from Prescott and Kuempel (19). Cells were grown at 41 C in M9 glucose medium, supplemented with 20 μg of thymine per ml and 10 μg of Casamino Acids per ml, to an A_{450} of about 0.3. The culture was starved for amino acids for 2 h in the presence of thymine (20 μg/ml). It was then filtered, washed, and suspended in M9 glucose medium supplemented with 2 μg of [^{3}H]thymine (final specific activity 10 C/mimol) and Casamino Acids. One-milliliter samples of this culture were pulse labeled for 2.5 min with 0.1 ml of [^{3}H]thymine (final specific activity 10 Ci/mmol) and min after the addition of thymine. The pulse was terminated by dilution into cold TV-CN buffer [0.01 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 8.1, 0.01 M ethylenediaminetetraacetate, 0.01 M NaCl]. Spheroplasts prepared according to Kuempel (9) were lysed and the DNA was spread on subbed glass microscope slides (10). The slides were dipped in melted Kodak NTB3 photographic emulsion and were exposed for 1 to 2 months at room temperature. They were developed in Kodak D-11 developer for 2 min at 20 C and fixed with Kodak F5 fixer.

RESULTS

Synchronous replication in a *dnaA*/R.100.1 strain at 30 C. The strain MUT26 (*dnaA*/R.100.1) was synchronized and the pattern of replication of the three markers, Mu-1::*ilv*, λ, and R.100.1, was followed at 30 C, a permissive temperature for the $dnaA_{ts}$ mutation (Fig. 2). It can be seen that, as in the parental strain previously analyzed in this laboratory (13), replication of *ilv* occurs immediately after the readdition of thymine. The replication of the R.100.1 plasmid begins simultaneously with that of the chromosome and continues for more

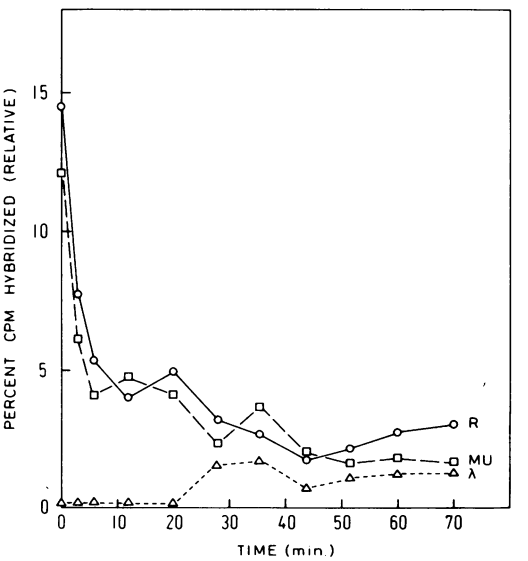

FIG. 2. *Replication pattern of MUT26 after synchronization at 30 C. The data were calculated as described in Materials and Methods. Replication was initiated at 0 min by the addition of 10 μg of thymine per ml.*

than 8 min. This is followed, 25 to 40 min later, by replication of the λ prophage. The peak of λ replication occurs at approximately 35 min, slightly later than in the parental strain at 37 C, a finding which is consistent with the effect of temperature on the replication velocity (18; Silver and Chandler, unpublished data).

Synchronous replication in integratively suppressed Hfr's. Similar experiments were conducted at the nonpermissive temperature of 42.5 C ("nonpermissive" refers to the *dnaA*$_{ts}$ allele and not to the strain itself) on strains LC2633 and LC2602 (Fig. 3 and 4, respectively).

Replication of the integrated R factor in both these strains occurs immediately after readdition of thymine to the growth medium. The kinetics of this replication are very similar to those observed in the R$^+$ parental strain at 30 C (Fig. 2). The most striking feature of the results shown in Fig. 3 and 4, however, is the absence of the characteristic early wave of replication from the normal chromosome origin and through the prophage Mu-1::*ilv*, which was found in the parental strains MUT26 (Fig. 2) and CB0129 (13). In the case of LC2633 (Fig. 3), no significant replication of the λ prophage is observed during the 60-min period covered by this experiment, while replication of the Mu-1::*ilv* prophage, situated close to the chromosome origin, begins at about 20 to 30 min and continues until at least 60 min. Under the same

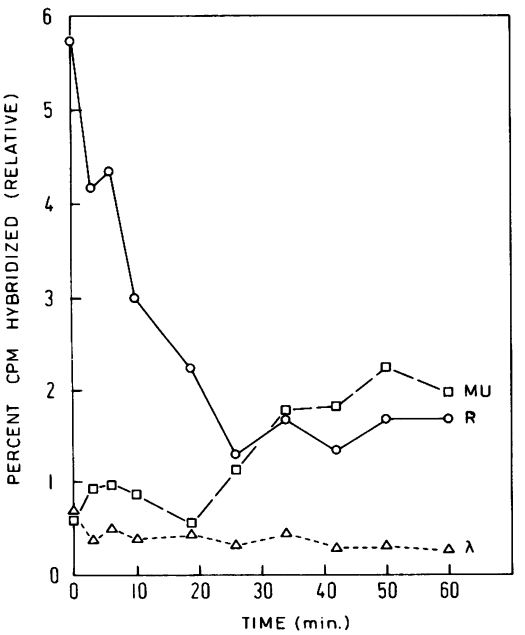

FIG. 3. *Replication pattern of LC2633 after synchronization at 42.5 C. See legend to Fig. 2.*

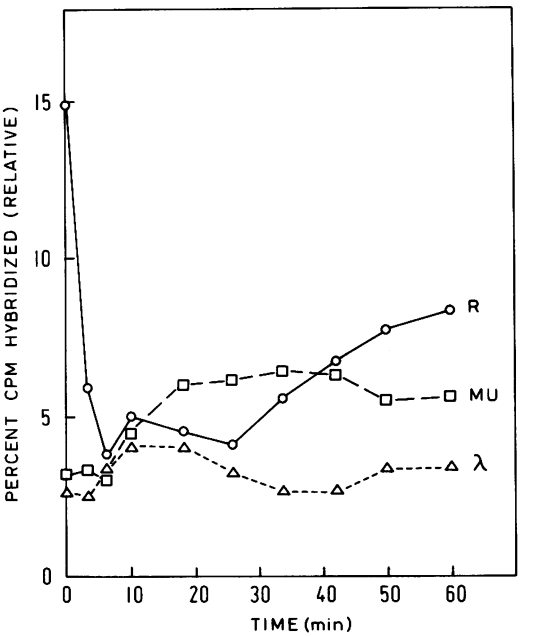

FIG. 4. *Replication pattern of LC2602 after synchronization at 42.5 C. See legend to Fig. 2.*

conditions, replication of the Mu-1 prophage in LC2602 occurs slightly earlier than in LC2633 and, in addition, a small peak of λ replication is seen (Fig. 4). The replication of λ occurs much earlier in LC2602 than in an R$^-$ strain at 37 C (13) or than in MUT26 at 30 C. The results shown in Fig. 4 also indicate that replication from R may proceed in both directions around the chromosome since R.100.1 is integrated at about 9.5 min on the *E. coli* genetic map (see Fig. 1) and both Mu-1 and λ appear to be replicated soon after the replication of R.

A pattern of replication similar to that obtained at 42.5 C is observed at 30 C for LC2633 (Fig. 5). Again, as for the culture grown at 42.5 C, LC2633 exhibits no replication of the λ prophage within the time limits of the experiment and a very broad timing in Mu-1 replication. The behavior of LC2602 at 30 C has not been investigated.

These experiments have been repeated several times with similar results. Comparable results have also been obtained with a third R.100.1 suppressed strain (unpublished data).

Control experiments. In each of the experiments concerning Hfr strains, 20 to 50 isolated colonies were tested for their ability to transfer chromosomal markers. In each case, all were Hfr with the correct transfer properties. In no case did we find transfer of resistance to chloramphenicol or streptomycin; this shows that R.100.1 had not reverted to the plasmid state.

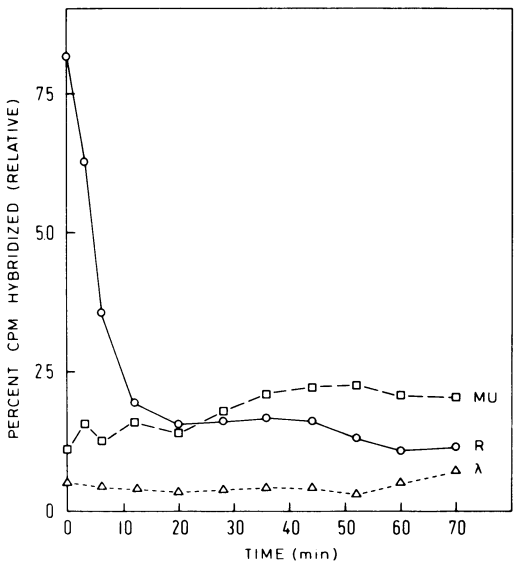

FIG. 5. *Replication pattern of LC2633 after synchronization at 30 C. See legend to Fig. 2.*

As a further control, we have analyzed cleared lysates of selected cultures, by centrifugation in ethidium bromide-cesium chloride gradients (5), for the presence of closed circular molecules exhibiting the same size in the electron microscope as R.100.1 (33 μm under the conditions used in this laboratory) or the RTF-TC component (26 μm). We were unable to detect such molecules in the electron microscope. We did, however, observe a peak in the gradients corresponding to closed circular DNA in LC2633. When fractions from this peak were analyzed by electron microscopy, a small closed circular plasmid was observed. Measured against λ DNA (17.3 μm, reference 3) as a standard, it had a contour length of 8.2 μm. The size of this molecule suggested that it could represent the r-determinant of R-100.1 (8.3 ± 0.2 μm, reference 17). Further analysis has confirmed this notion (in preparation). Identical results were obtained in our hybridization experiments whether DNA from R.100.1 or from RTF-TC, which contains few, if any, r-determinant sequences, was used on the filter; thus the presence of the plasmid did not affect our results, and we are confident that the R replication observed represents replication from the integrated plasmid.

Autoradiography. Cultures of LC2633 were allowed to initiate replication, after a period of amino acid starvation, in the presence of low-specific-activity [^{3}H]thymine, were pulse labeled later with high-specific-activity [^{3}H]thymidine, and autoradiographed.

Chromosomes that replicate in a bidirectional manner and have initiated replication in the presence of the low-specific-activity thymine show a low-grain density track (representing DNA synthesized during this first period), flanked by two regions of high-grain density (representing DNA synthesized during growth in the high-specific-activity thymidine). Unidirectionally replicating molecules would be expected to have a region of low-grain density with only one region of high-grain density, a pattern which would also be expected for bidirectionally replicating molecules which had been sheared during treatment.

Among the grain tracks found during this experiment, we observed patterns characteristic of both bidirectionally and unidirectionally replicating molecules. Two examples of bidirectionally replicating molecules are shown in Fig. 6. However, it was not possible to obtain an estimate of the relative frequency of these forms since the amino acid starvation regime did not result in the complete cessation of DNA synthesis before growth in radioactive medium (see Discussion). Although we can conclude that replication is bidirectional for at least a fraction of the population, we cannot determine whether it can occur in a unidirectional mode as well.

An analysis of the lengths of the heavily labeled extremities of bidirectionally replicating molecules is shown in Fig. 7. This figure represents the results of two experiments in which growth in low-specific-activity thymine was allowed to continue for 15 or for 20 min and was followed by a pulse of high-specific-activity thymidine for 2.5 min. The results are presented as a plot of the shortest labeled extremity of a molecule versus its longest extremity. It can be seen that in general the molecules are symmetric with regard to these extremities. This indicates that, in a given molecule, both replication forks proceed at a comparable speed. The data also imply considerable spread in fork velocity between chromosomes.

In all of the molecules analyzed which showed a bidirectional replication pattern (44 molecules), the total length of the labeled region (i.e., low-density and high-density grain tracks) was greater than 33 μm, the length of the autonomous R factor; in 11 cases the length was greater than 100 μm. If these grain patterns represent initiations of replication within the R factor, this result indicates that replication continues well into the chromosome in both directions. To estimate errors due to stretching of the DNA molecules, labeled DNA from bacteriophage λ was used. The length of λ DNA measured by electron microscopy is ap-

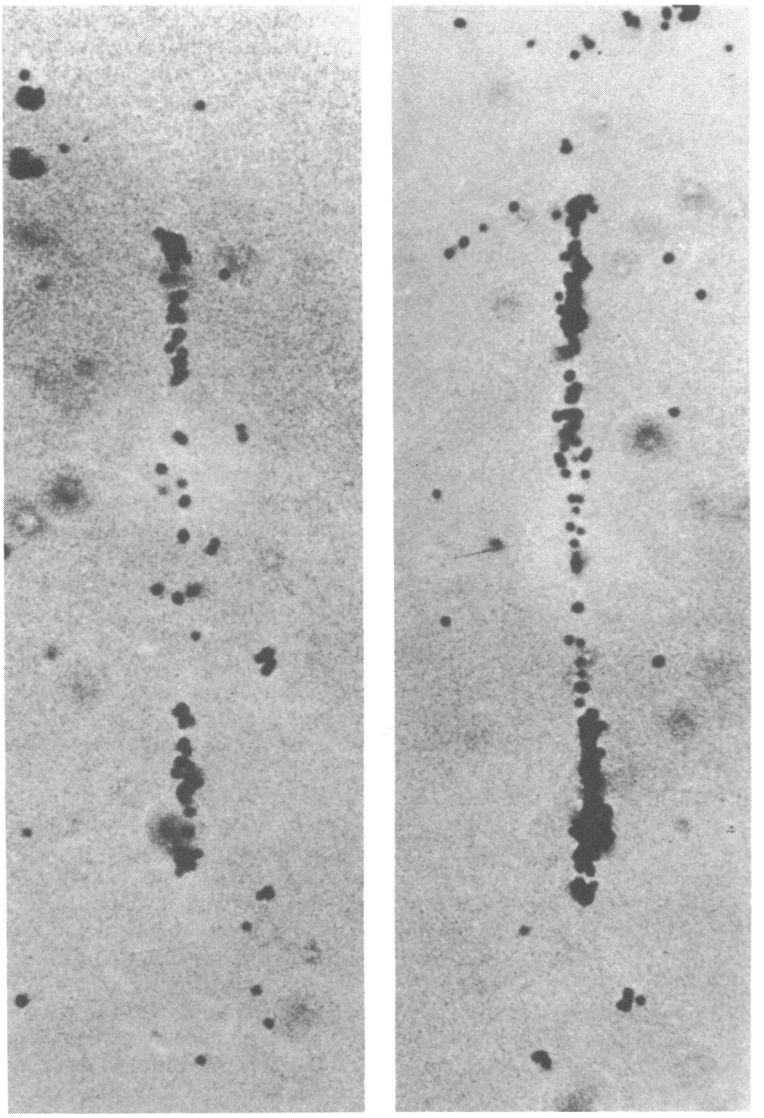

FIG. 6. *Autoradiographs of two newly initiated DNA molecules. The two molecules shown in this figure are taken from an experiment in which initiation of chromosome replication in LC2633 was allowed to continue for 20 min in the presence of low-specific-activity thymine before a 2.5-min pulse of high-specific-activity thymidine.*

proximately 17.3 μm (3). The bacteriophage DNA was mixed with unlabeled spheroplasts and autoradiographed in exactly the same manner as was the labeled bacterial DNA. Measurement of the length of the resulting grain patterns yielded a value of 14.2 μm with a standard deviation of 33%.

DISCUSSION

In the two integratively suppressed strains which we have studied, the synchronization procedure carried out at the nonpermissive temperature of 42.5 C results in the initiation of a round of replication from the integrated R factor in the majority of the chromosomes. In contrast to the parental *dnaA*/R$^+$ strain (MUT26), at 30 C, there is little, if any, initiation of chromosome replication at the normal chromosome origin as defined by MU::*ilv*.

One difficulty which we have experienced in the interpretation of these results stems from the ability of the two Hfr strains to synthesize DNA during the amino acid starvation. We have found that, in contrast to both the paren-

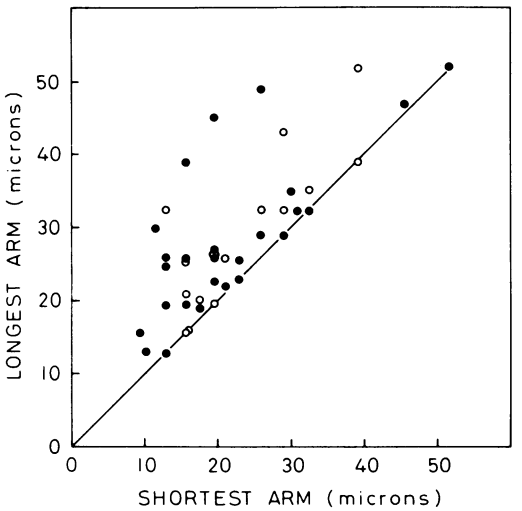

FIG. 7. *Relative lengths of the heavily labeled extremities of newly initiated chromosomes. In this figure, the length of the longest heavily labeled extremity, obtained from the photographs such as those shown in Fig. 6, is plotted against the length of the other extremity. The curve expected for an equal rate of travel of both replication forks is also shown. The open and closed circles represent measurements on chromosomes which had been allowed to continue replication in low-specific-activity thymine for 20 and 15 min, respectively.*

tal strain (MUT26) at 30 C and to a related *dnaA*$^+$ strain growing at 42.5 C, both LC2633 and LC2602 continue linear DNA synthesis for about 6 or 7 h and synthesize up to 180% of the DNA present at the time of removal of amino acids. Autoradiograph experiments and hybridization experiments (data not shown) revealed that, following the period of amino acid starvation used in the synchronization procedure, a large fraction of the population was still replicating its DNA but that this replication was random; i.e., the prophages Mu-1 and λ and the integrated plasmid were being replicated to a similar extent. Thus this continued replication must contribute to the background during a synchronous round of replication since there were replication forks remaining on the chromosome after the synchrony procedure. Upon readdition of thymine to the growth medium, these replication forks continue to synthesize DNA (see for example the initial levels of λ replication in LC2602, Fig. 4). However, since they are distributed over the entire length of the chromosome, they provide a general background of random replication and do not obscure the major peaks of synchronized replication.

The data obtained for LC2633 show that, at 30 C (a permissive temperature for the $dnaA_{ts}$ mutation), synchronization results in initiation at the R site rather than at the normal origin. From the results obtained with MUT26 at 30 C (Fig. 2), the absence of any observable replication from the normal origin is surprising. It suggests that in this strain, even at 30 C, the integrated R factor in some fashion prevents replication from the normal origin.

The initiation of a round of replication from the R100.1 origin at 30 C, as well as at 42 C, dure. Data (in preparation) on gene frequency at both temperatures in exponential cultures indicate, however, that the normal replication origin can be used at 30 C but is used much less frequently, if at all, 42 C.

In both LC2602 and LC2633, MU::*ilv* is located in a similar position relative to R and to the direction of chromosome transfer. The data obtained for LC2602 and, in particular, the early wave of λ replication show that, at least in a fraction of the cells, replication occurs in the opposite direction as well. This was confirmed by autoradiography. Similar conclusions have been reached by J. Louarn and J.-M. Louarn using related strains but different methods (in preparation).

The peaks of Mu-1 replication in LC2633 and of λ and Mu-1 in LC2602 (Fig. 3 and 4) are extremely broad. This suggests that the rate of travel of individual replication forks in this system is quite varied, much more so than in a related wild-type strain at 37 C (13). The results obtained by analysis of autoradiographs (Fig. 7) also support this idea. This wide variation in replication fork velocity is not seen in the parental R$^+$ strain (MUT26). The fork velocity in MUT26 as measured by the time between Mu-1::*ilv* and λ replication, appears to be close to that expected for wild-type strains growing at 30 C (18; Silver and Chandler, unpublished data). Because of this heterogeneity in the Hfr strains, it is difficult to determine the average fork velocity. The maximum fork velocity, however, can be estimated from the time when label specific for a marker begins to rise above the background level. Thus, in LC2633 (Fig. 3), the wave of replication initiated at R reached Mu-1, 15 map units away, between 20 and 25 min after initiation. In a related wild-type strain at 37 C, a temperature at which fork velocity is normally slower than at 42.5 C, the equivalent distance between the normal origin and *thr* (about 15 map units) was covered in 10 to 12 min (13). Thus fork velocity in LC2633 is at least half that found in similar experiments in the parental strains. The maximum fork velocity observed for LC2602 (Fig. 4) appears,

on the other hand, to be much closer to the wild-type situation.

These differences in replication fork properties between the parental strains and the integratively suppressed Hfr's could reflect differences in the replication fork itself during integrative suppression, or differences in the physiology of the cell, e.g., permeability, nucleotide pools, etc.

In summary, the data shown here demonstrate that, in *dnaA* strains integratively suppressed by an R factor, R.100.1, and synchronized by amino acids and thymine starvation, the first round of replication occurring after the restoration of thymine, at 42.5 C, is initiated at the site of integration of the R factor rather than at the normal origin. Replication from R, rather than from the normal chromosome origin, was also observed in one of the Hfr's synchronized at 30 C. Such changes in origin have no lethal effect on the cells that can grow at 42.5 C or at 30 C. They have also been observed in a *dnaA*$^+$ F-Hfr under similar conditions (M. Chandler, L. Silver, Y. Roth, and L. Caro, J. Mol. Biol., in press).

The pattern of chromosome replication in integratively suppressed strains during exponential growth at 30 and 42 C will be considered in a subsequent communication.

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ADDENDUM

We recently measured the Mu/λ ratio in preparations of DNA from a culture of MUT26 that had been starved for amino acids at 30 C for 3 h. Contrary to our expectations, this ratio, when compared with that obtained from DNA of strain MX213, which had been treated in an identical manner (and whose Mu/λ ratio is defined as unity; M. Chandler et al., J. Mol. Biol. 94:127–132, 1975), yielded a value of 1.97. Since MUT26 synthesizes the expected amount of DNA during amino acid starvation and in all other respects behaves in a manner identical to that of MX213 during this procedure, the result indicates the presence of two copies of the Mu prophage in MUT26 and, therefore, in strains LC2602 and LC2633. When MUT26 is transduced to *ilv*$^+$, 90% of the *ilv*$^+$ transductants become sensitive to Mu-1, demonstrating that both copies of the prophage are located very close together in the *ilv* region.

This finding in no way affects the interpretation we have placed on our results since, in the treatment of the data, no correction was made for the differences in molecular weights or the hybridization efficiencies of the various DNA species.

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