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Synthesis of Linear Multimers of OriC and pBR322 Derivatives in *Escherichia coli* K-12: Role of Recombination and Replication Functions

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Inactivation of RecBCD nuclease (exonuclease V) and SbcB nuclease (exonuclease I) in *Escherichia coli* K-12 diverts most of plasmid replication activity from circular monomer production to the synthesis of linear multimers. Linear multimer synthesis has been demonstrated in plasmids of diverse origins and copy numbers, including *E. coli* minichromosomes. The effect of *dnaA*, *dnaB*, *recF*, and *recJ* mutations on the rate of linear multimer synthesis in *sbcB* cells after *gam* inactivation of RecBCD nuclease was investigated. Results are consistent with the hypothesis that homologous recombination, but not activities at the plasmid origin of replication, is involved in initiation of linear multimer synthesis.

Replication of ColE1-type plasmids is initiated at the 3′ OH end of an RNA primer. After initiation, it proceeds unidirectionally by a theta-like mode of replication to yield circular plasmid monomers (26). Copy number is controlled at the primer formation stage in this system by a small RNA species, RNA I, which is transcribed from the DNA strand opposite to that encoding the primer (44).

Recently, we (11) demonstrated another mode of replication for high-copy-number plasmids. This activity yields linear multimers rather than circular monomers. It is inhibited by RecBCD exonuclease and exonuclease I (*sbcB* enzyme) and depends on a functional RecA protein. Unlike normal replication, linear multimer synthesis is insensitive to plasmid-copy-number control mechanisms. Thus, inactivation of RecBCD nuclease in *sbcB* mutants leads to a rapid accumulation of plasmid linear multimers in the cell (11).

Synthesis of linear plasmid multimers in *recB recC sbcB* mutants has been observed with derivatives of ColE1-type plasmids and with λdv (11). It is of interest to determine whether it also affects other replicons such as low-copy-number plasmids and *Escherichia coli* minichromosomes. In this paper we address this question and determine the dependence of this mode of replication on functions which are involved in plasmid replication and recombination in wild-type cells.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used are listed in Table 1. Plasmids carrying cultures were grown in L broth (32) supplemented with the appropriate antibiotics (100 μg of ampicillin per ml and 20 μg of kanamycin per ml). Cultures of temperature-sensitive strains and of mutants carrying pSF117 or pSF119 were grown at 28°C. *c*I857 from pSF117 or pSF119 was inactivated by a temperature shift to 37°C. DNA replication in *dnaA46* or *dnaB558* mutants was arrested by a temperature shift to 42°C.

Plasmids. Plasmids used in this study are listed in Table 2. pSB5 was constructed by S. Broido in this laboratory. It consists of a 4.4-kilobase *Hin*cII fragment of pMC903 (8) which includes the P15A origin of replication and the kanamycin resistance gene. pSF117 and pSF119 were a gift from J. Hays. pSF117 carries a short form of the *gam* (*gamS*$^+$) gene of bacteriophage λ and a *c*I857 gene (19). pSF119 is a derivative of pSF117 carrying a *gamS201* mutation (19). pOC81 was a gift from W. Messer. It contains an *Hin*cII fragment (3837 to 1492) of pCM959 (34) and a kanamycin resistance gene.

Preparation of DNA. Plasmid DNA was prepared by cesium chloride-ethidium bromide density gradient centrifugation of clear lysates (10). Total DNA (plasmid and chromosomal) was prepared from cells at the log phase as described by Gillen et al. (23).

Southern hybridization analysis of plasmid molecular forms. Total DNA preparations (2 μg per lane) were subjected to electrophoresis for 16 h at 1.5 V/cm in TBE buffer on a 0.7% agarose gel (33). DNA was denatured, transferred to nitrocellulose filters, and hybridized to a ^{32}P-labeled DNA probe by the Smith and Summers modification (41) of the Southern procedure (40). Labeled pSF117 was used as a probe for the detection of pSF117 or pSF119 molecular species. An *Xho*I-*Bam*HI fragment of pSB5 which includes the *kan* gene was used for detection of pSB5 and pOC81 in total DNA preparations. This fragment does not cross-hybridize with pSF117. Radioactive labeling of the probes was done by nick translation (33). After hybridization, autoradiograms were scanned with a Helena Quikscan microdensitometer, and the ratio of linear multimers to circular monomers was determined by weighing the corresponding peaks of the tracings.

Enzymes. Restriction endonucleases and DNA polymerase were purchased from New England BioLabs, Inc. (Beverly, Mass.). Enzymatic reactions were performed as directed by the supplier. For partial hydrolysis by restriction enzymes, 0.1 unit/μg of DNA was used, and incubation was for 1 h. RecBCD enzyme (exonuclease V) was a gift from Andrew F. Taylor and Gerald R. Smith. Digestion with exonuclease V was performed by the method of Lackey and Linn (28).

Determination of relative rates of DNA synthesis. Samples (2 ml) of exponentially grown cultures were pulse-labeled with 10 μCi of [*methyl*-^{3}H]thymidine per ml (2 Ci/mmol) in the presence of 200 μg of adenosine per ml for 5 min. A 0.1-ml sample of the mixture was added to 3 ml of 10%

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TABLE 1. *E. coli* strains

Strain	Genotype								Source or reference
	recB	*recC*	*sbcB*[a]	*recF*	*recJ*	*dnaA*	*dnaB*	Others	
JC5519	*21*	*22*	+	+	+	+	+	—[b]	45
JC7623	*21*	*22*	*15*	+	+	+	+	—[b]	42
JC11451	+	+	*15*	+	+	+	+	—[c]	A. Templin
AC113	+	+	*15*	+	*284*::Tn*10*	+	+	—[b]	This work[d]
AC116	+	+	*15*	*143*	+	+	+	—[b]	This work[e]
AC120	+	+	*15*	+	+	*46*	+	—[b]	This work[f]
AC123	+	+	+	+	+	*46*	+	—[b]	This work[g]
AC129	+	+	*15*	+	+	+	*558*::Tn*10* *Δ16Δ17* Kan[r]	—[b]	This work[h]

[a] All *sbcB15* mutants listed in this table have or are likely to have *sbcC201* (30).
[b] All strains are derived from AB1157 (3). Other mutations are: *thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*.
[c] JC11451 is a *sup*$^+$ derivative of JC7689 (27). Other mutations are as in footnote *b*.
[d] AC113 is a Tet[r] transductant from JC11451 by PI-JC12123 (31).
[e] AC116 is a Tet[r] transductant from JC11451 by PI-JC12334 (38).
[f] AC120 is a Tet[r] temperature-sensitive derivative of JC11451, constructed by transduction with PI-CG642. CG642 is a *dnaA46 tna*::Tn*10* strain constructed by C. Georgopoulos.
[g] AC123 is a Tet[r] temperature-sensitive derivative of AB1157 (3), constructed by transduction with PI-CG642.
[h] AC129 is a Kan[r] temperature-sensitive derivative of JC11451, constructed by transduction with PI-AC122. AC122 is a *dnaB558*::Tn*10Δ16Δ17* kan[r] (16) strain, constructed by transduction of CG53 by PI-CG1088. CG53 carries a *dnaB558* (21) mutation. CG1088 is a *dnaB*$^+$::Tn*10Δ16Δ17* kan[r] strain. Both strains were a gift from C. Georgopoulos.

trichloroacetic acid, and incorporation of [^{3}H]thymidine into acid-precipitable material was determined.

RESULTS

Synthesis of linear multimers by *E. coli* minichromosome. The plasmid pOC81 is an OriC derivative carrying 2,346 base pairs of *E. coli* chromosome origin of replication and a kanamycin resistance marker (35). The distribution of the molecular species of this plasmid in *recB21 recC22* (JC5519), *sbcB15* (JC11451), and *recB21 recC22 sbcB15* (JC7623) mutants of *E. coli* K-12 was determined by Southern hybridization (Fig. 1). Most of the pOC81 DNA in *recB21 recC22* and *sbcB15* mutants was associated with supercoiled monomers. On the other hand, in *recB21 recC22 sbcB15* cells, most of the pOC81 DNA comigrated with linear multimers of high-copy-number plasmids and with chromosomal DNA. The noncircular structure of pOC81 DNA, which is associated with this band, was demonstrated, as previously done (11), by its susceptibility to digestion by RecBCD exonuclease (Fig. 2A). To confirm that the slow-migrating material consists of pOC81 concatamers, we digested total DNA preparations with *Pst*I, which has a unique site on pOC81, and subjected them to electrophoresis and hybridization. While complete digestion with *Pst*I yielded a single product (linear monomer), partial digestion yielded products that were also hybridizable and that corresponded in their mobility to pOC81 linear oligomers (Fig. 2B). The electrophoretic mobility of the hybridization band, its susceptibility to recBCD exonuclease, and its restriction endonuclease products suggest that pOC81, like high-copy-number plasmids, serves as a substrate for linear multimer synthesis in *recB recC sbcB*

TABLE 2. Plasmids

Name	Source or reference	Native replicon	Derived plasmid	Reference for derived plasmid
pOC81	35	*E. coli* chromosome	pCM959	34
pSB5	S. Broido	P15A	pACYC177	14
pSF117	19	pMB1	pBR322	7
pSF119	19	pMB1	pBR322	7

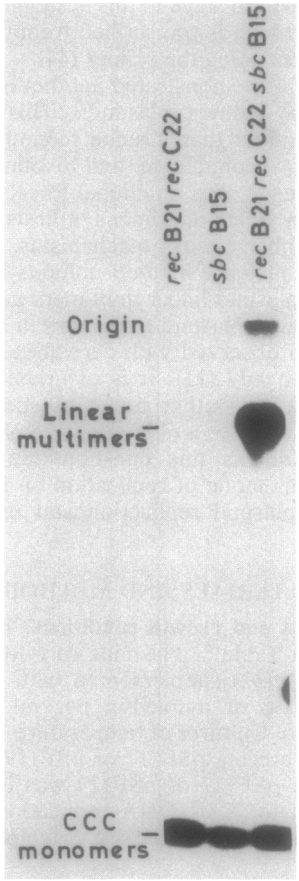

FIG. 1. Southern analysis of pOC81 DNA in *E. coli* strains. Total DNA preparations of cells of the designated genotype harboring pOC81 were subjected to Southern hybridization with a radiolabeled *Xho*I-*Bam*HI pSB5 fragment as a probe. The locations of pOC81 covalently closed monomers (ccc monomers) and of the linear multimers of pSB5 (linear multimers) are indicated. (Chromosomal DNA comigrates with linear multimers in this system.)

mutants. A fraction of OriC DNA in *recB recC sbcB* cells was detectable at the electrophoretic origin. A similar band was observed when ColE1 derivatives were propagated in the same mutant (11). The molecular nature of this material has not yet been determined.

Inhibition of RecBCD enzyme by thermal activation of *gam* function in *sbcB* mutants harboring plasmid pSF117 results in the accumulation of pSF117 linear multimers. When *sbcB* mutants are cotransformed with pSF117 and another compatible plasmid, accumulation of linear multimers of both plasmids is observed under the same conditions (11). The rate of accumulation of pOC81 linear multimers was compared in this system with that of pSB5, a Km^r derivative of plasmid P15A (Fig. 3). Cultures of an *sbcB15* mutant (JC11451) harboring pSF117 and either pSB5 or pOC81 were grown at 28°C. Gam activity was thermally induced by transferring the cultures to 37°C. Samples were taken at various time intervals after temperature shift, and total DNA preparations were analyzed by Southern hybridization with the kanamycin resistance gene as a probe (Fig. 3). An increase in the amount of pOC81 linear multimers was observed after thermal activation of Gam synthesis in *sbcB15* cells. This increase depends on *gam* activity, since it is not observed when pSF117 is substituted by pSF119 in this system (data not shown). The kinetics of linear multimer accumulation by pOC81 plasmids differed from that of pSB5. While linear multimers of pSB5 were observed as early as 15 min after temperature shift, linear multimer formation by pOC81 was observed only after a 1-h lag, and rate of increase was lower than that of pSB5 accumulation.

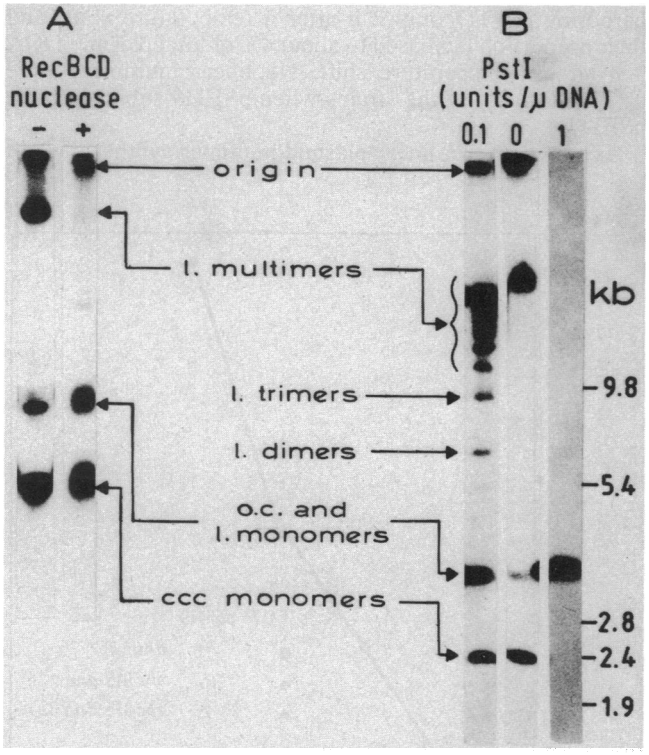

FIG. 2. Digestion of pOC81 DNA in *recB21 recC22 sbcB15* cells by RecBCD nuclease and *Pst*I. Total DNA preparations of *E. coli* JC7623 cells harboring pOC81 were subjected to digestion by RecBCD nuclease (A) and to partial or complete digestion by *Pst*I (B). Electrophoresis was on a 0.7% agarose gel for RecBCD nuclease-digested DNA and on a SeaKem LE 0.4% agarose gel for *Pst*I-digested DNA. Electrophoretically separated pOC81 DNA fragments were visualized by Southern hybridization. The lengths of hybridizing fragments were determined by using molecular weight markers as described previously (33). Location of pOC81 covalently closed circular (ccc) monomers, open circular (o.c.) monomers, linear monomers (l.monomers), linear oligomers (l.multimers), and molecular length standards are indicated. kb, Kilobases.

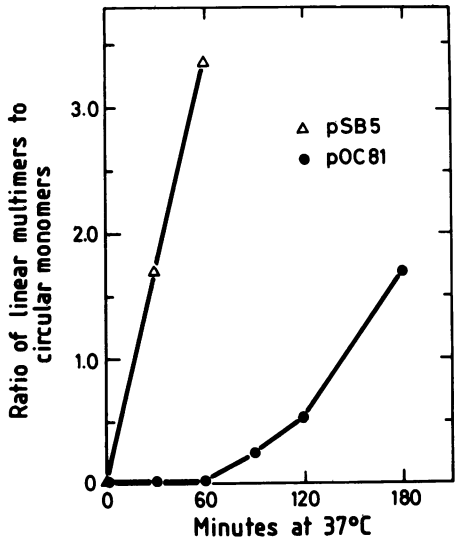

FIG. 3. Accumulation of pSB5 and pOC81 linear multimers after derepression of $gamS^+$ in *sbcB15* cells. Samples of exponential-phase cultures of JC11451 cotransformed with pSF117 and either pSB5 or pOC81 were transferred from 28 to 37°C for the indicated period. Total DNA preparations (2 μg) were subjected to Southern hybridization analysis with an *Xho*I-*Bam*HI pSB5 DNA fragment as a probe. The hybridization autoradiograms were analyzed by microdensitometer scanning, and the ratio of linear multimers to monomers was determined by weighing the corresponding peaks from photocopies of the tracings.

Dependence of linear multimer synthesis on *dnaA* and *dnaB* activities. To relate the replication process which yields linear multimers to normal plasmid replication, we tested the role of two functions which are involved in plasmid replication: *dnaA*, which is involved in initiation of plasmid replication at the origin (20), and *dnaB*, which is essential for any replication to occur (29).

DnaA protein is a positive regulator of replication initiation at the chromosomal origin (20). Thus, synthesis of OriC plasmid DNA is arrested after transfer of *dnaA*(Ts) mutants to the restrictive temperature (45). To determine the effect of *dnaA* inactivation on linear multimer synthesis by an OriC replicon, *sbcB15 dnaA46* double mutants (AC120) harboring pSF117 and pOC81 were grown at 28°C and transferred to 42°C for the indicated time. Total DNA preparations were subjected to Southern hybridization, and the ratio of linear multimers to monomers was determined (Fig. 4). While the rate of total DNA synthesis declined after transfer to the restrictive temperature, the synthesis of pOC81 linear multimers was not affected. As in *sbcB* ($dnaA^+$) cells, pOC81 linear multimers were detectable 60 min after temperature shift, and their proportion increased thereafter.

Synthesis of pBR322 derivatives proceeds after *dnaA* inactivation (13). However, the rate of synthesis is reduced (1, 37), and the mode of synthesis differs from that in $dnaA^+$ cells. Replication mainly occurs by a rolling circle mechanism (1) and is resistant to rifampin (1, 37). Results presented in Fig. 5 indicate that *dnaA* activity is not involved in the synthesis of pSF117 linear multimers. Multimer synthesis of

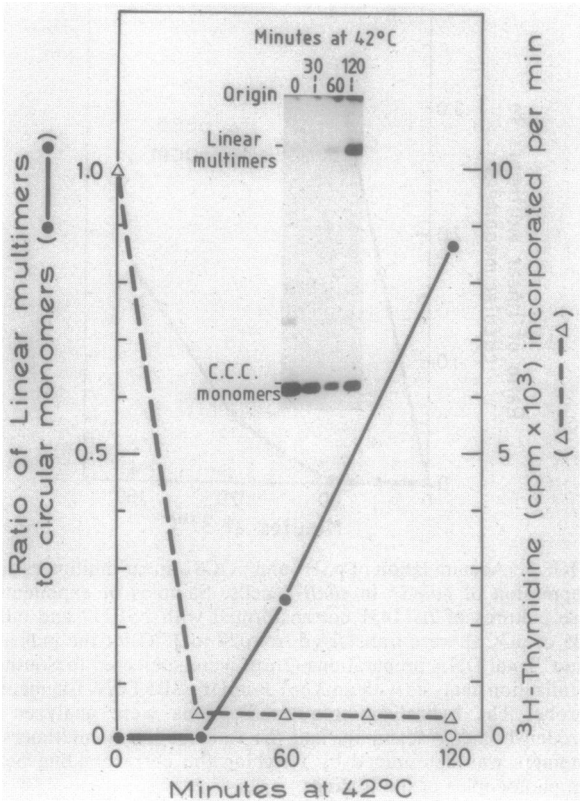

FIG. 4. Accumulation of pOC81 linear multimers in *sbcB15 dnaA46* cells at 42°C. Exponential cultures of AC120 harboring pSF117 and pOC81 were transferred from 28 to 42°C for the indicated period. Total DNA preparations were subjected to Southern hybridization (insert), and the ratio of pOC81 linear multimers to circular monomers was determined as described in the legend to Fig. 3. The rate of incorporation of [^{3}H]thymidine into acid-insoluble material was determined in samples taken at the indicated time and incubated in the presence of [^{3}H]thymidine for 5 min. c.c.c., Covalently closed circular.

pSF117 plasmids in *sbcB15 dnaA46* cells was initiated after the temperature shift and proceeded at a rate similar to that observed in *sbcB* (*dnaA*$^+$) hosts. As in *dnaA*$^+$ cells, accumulation of linear plasmid multimers in *dnaA46* cells depended on inactivation of both RecBCD exonuclease and exonuclease I. Replication was not observed in a *dnaA46* (*sbcB*$^+$) (AC123) strain or when pSF119 substituted for pSF117.

While DnaA protein is specifically involved in initiation of replication at the origin, DnaB protein is part of the primosome system (2) and acts as a helicase (29), and as such, it is involved directly in the propagation of the replication forks. The synthesis of linear multimers of pSF117 depended on *dnaB* activity (Fig. 5). Linear multimers were not observed when *sbcB15 dnaB558* double mutants (AC129) harboring pSF117 were incubated at 42°C. A Southern blot of a total DNA preparation of such cultures was indistinguishable from that of a culture harboring pSF119 and incubated at the same temperature.

Dependence of linear multimer synthesis on *recF* and *recJ* activities. The dependence of linear multimer synthesis on *recA* activity in a *recB recC sbcB* genetic background suggests that recombination is involved in this mode of DNA replication. However, the minor effect of *recJ* and *recF* mutations on the proportion of linear multimers in the same genetic background argues against this conclusion (11). While the presence of linear multimers in *recB recC sbcB recF* and *recB recC sbcB recJ* mutants indicates that their synthesis occurs regardless of *recJ* and *recF* genotypes, their proportion in these mutants does not necessarily reflect their rate of accumulation. This rate can be determined in a *sbcB* (*recB*$^+$) genetic background after thermal activation of the *gam* function of pSF117.

Cultures of *sbcB15* mutants (JC11451) and isogenic strains carrying *recF143* (AC116) or *recJ284* (AC113) mutations were transformed with pSF117 and grown at 28°C. Samples were transferred to 37°C for the indicated period, and total DNA preparations were analyzed for the ratio of linear plasmid multimers to plasmid circular monomers (Fig. 6A) and for the ratio of pSF117 plasmid DNA to chromosomal *Eco*RI fragments longer than linear plasmid monomers (Fig. 6B). As observed before (11), linear plasmid multimers were synthesized in *recJ* and *recF* mutants when both RecBCD nuclease and exonuclease I were inactivated. However, the rate of multimer synthesis was lowered by *recF* and *recJ* mutations. Although in *sbcB15* mutants harboring pSF117 the majority of the plasmid DNA population consisted of linear multimers at less than 1 h after a temperature shift, in an isogenic strain carrying a *recJ* mutation, this molecular species reached only 15% of total plasmid DNA after 1 h at 37°C. Linear multimers were detectable in *sbcB recF* cells harboring pSF117 only 2 h after a temperature shift, and their proportion increased to about 4% of total plasmid DNA 3 h after a temperature shift. No linear multimers were observed in any of the strains when pSF119 substituted for pSF117.

As stated above, linear plasmid multimer synthesis is not

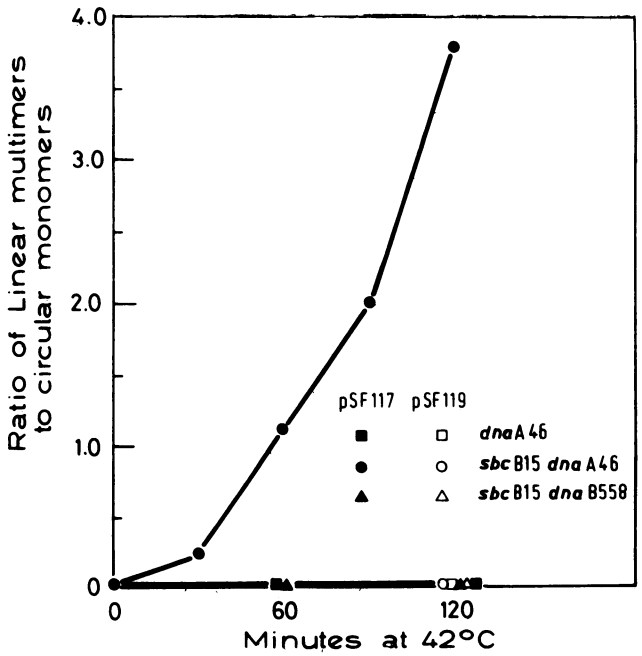

FIG. 5. Effect of *dnaA46* and *dnaB284* mutations on the accumulation of pSF117 linear multimers in an *sbcB15* genetic background. Cultures of the designated genotypes harboring pSF117 or pSF119 at the exponential phase of growth were transferred from 28 to 42°C for the indicated period. Total DNA preparations were subjected to Southern hybridization with radiolabeled pSF117 as a probe. The ratio of linear multimers to circular monomers was determined as described in the legend to Fig. 3.

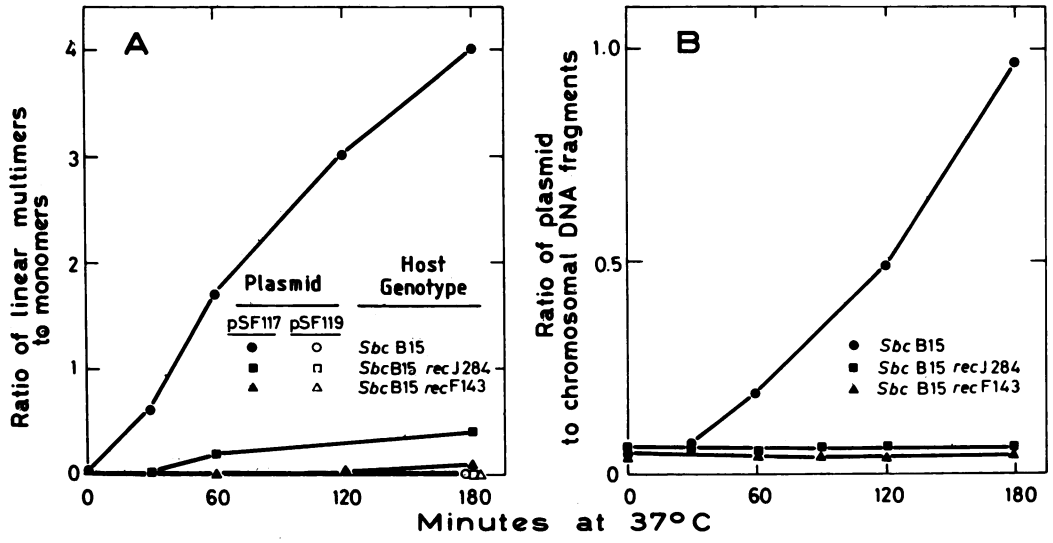

FIG. 6. Effect of *recF143* and *recJ284*::Tn*10* mutations on accumulation of pSF117 linear multimers in an *sbcB15* genetic background. Cultures of the designated genotypes harboring pSF117 or pSF119 at the exponential phase of growth were transferred from 28 to 37°C for the indicated period. The ratio of plasmid linear multimers to circular monomers was determined as described in the legend to Fig. 3. To determine the increase in the proportion of pSF117 in the cell after the temperature shift, equal amounts (3 μg) of DNA were digested with *Eco*RI to fragment the chromosomal DNA and to form monomers of the plasmid DNA and were subjected to agarose gel electrophoresis in the presence of ethidium bromide (33). The electrophoretograms were photographed under UV illumination, and the negatives were scanned with a microdensitometer. The ratio of linear plasmid monomers to chromosomal DNA fragments that were longer than the linear plasmid monomers was determined for each sample by weighing the corresponding areas of photocopies of the tracing (11).

effectively controlled by the plasmid-copy-number control mechanism. Therefore, activation of this mode of plasmid replication leads to an increase in the proportion of plasmid DNA in the cell. This increase can be followed in *sbcB* cells harboring pSF117 by electrophoresis of *Eco*RI-digested total DNA preparations in the presence of ethidium bromide, photography under UV illumination, and microdensitometer tracing of the negatives (11). While an increase in the ratio of plasmid to chromosomal DNA was observed in *sbcB15* cells harboring pSF117 after the temperature shift (Fig. 6B), no such increase was detectable in *sbcB15 recF143* or *sbcB15 recJ284* mutants harboring the same plasmid under the same conditions.

Results presented in this section indicate that linear multimer synthesis in *sbcB* cells after derepression of Gam protein depends on *recF* and *recJ* activities. These observations and the dependence of linear multimer synthesis on RecA activity in *recB recC sbcB* mutants (11) suggest that homologous recombination is involved in this mode of replication. However, we cannot rule out the possibility that *recJ* and *recF* mutations interfere with *gam* inhibition of RecBCD nuclease.

DISCUSSION

Inhibition of RecBCD nuclease leads to the activation of a DNA synthesis pathway that converts circular monomers into linear multimers. The similarity between this mode of replication and lambda phage DNA linear multimer synthesis led to the proposal that plasmids, like λ phage DNA (5, 18), replicate by a rolling circle mechanism after RecBCD nuclease inactivation (11). We have demonstrated that this mode of DNA synthesis affects several replicons of diverse origins, such as high-copy-number plasmids (11), *E. coli* minichromosomes (this report), and derivatives of medium-copy-number (pSC101) and low-copy-number (mini-F) plasmids (A. Cohen and Z. Silberstein, unpublished data). We therefore propose that circular replicons can serve as substrates for either one of two types of replication. One type is initiated at the origin, yields circular monomers, and is controlled by the functions of the replicon. The other type yields linear multimers, and its activity is not effectively controlled.

The observation that all replicons which have been tested, including minichromosomes, can serve as substrates for linear multimer synthesis has several interesting implications. Most notable is the possibility that under certain genetic and physiological conditions such activity affects the chromosome.

Linear DNA molecules, which are present in *recB recC*, *recB recC sbcA*, or *recB recC sbcB* mutants after conjugation, may prime a rolling circle type of synthesis at a homologous site on the chromosome. Such a reaction may lead to the production of transcribable, but not inheritable, recombination intermediates in *recB* mutants (6). It may also be involved in the production of recombinants via the RecE or RecF recombination pathway (4, 9) in *recB recC sbcA* or *recB recC sbcB* mutants, respectively. A role for linear DNA ends in plasmid recombination in *recB recC sbcA* mutants, but not in wild-type cells, has recently been proposed (43).

RecBCD nuclease appears to play a major role in determining the mode of replication activity in the cell. In wild-type cells in which this nuclease is active, all or most replication activity is directed toward the production of circular monomers. Inactivation of RecBCD nuclease by mutations or by phage activity diverts replication toward the production of linear multimers. Linear multimer production in a *recB recC* genetic background is greatly enhanced by inactivation of exonuclease I or by activating 5′ exonucleases such as exonuclease VIII (11) or lambda exonuclease

(18). The role of these exonucleases in determining plasmid modes of replication will be discussed elsewhere (A. Cohen, S. Maor, and Z. Silberstein, manuscript in preparation).

The dependence of plasmid linear multimer formation on *dnaB* activity, but not on *dnaA*, indicates that DNA synthesis is involved in this process but that initiation of this mode of replication differs from that of normal plasmid replication. Differences in mechanisms of initiation between normal replication and linear multimer synthesis were also suggested by the insensitivity of the latter activity to plasmid-copy-number control systems (11).

Replication may be initiated independently of the activities at the origin by alternative mechanisms, such as priming by recombination (15, 18, 36) from a 3′ OH end at a strand break (22) or at alternative *ori* sites on the replicon (17). Plasmid recombination in *E. coli* depends on *recA*, *recF*, and *recJ* activities (12, 24, 25). The dependence of linear multimer synthesis on *recA* activity and its insensitivity to *lexA3* mutations in *recB recC sbcB* cells suggested a role for recombination in this mode of plasmid replication (11). This proposal is further substantiated by the observation that linear multimer synthesis after RecBCD nuclease inactivation in *sbcB* cells depends on *recF* and *recJ* activities.

Although recombination appears to be an effective mechanism for initiation of plasmid linear multimer synthesis, the residual activity which is observed in *recB recC sbcB* strains carrying *recA*, *recF*, or *recJ* mutations (11) and in *recJ sbcB* and *recF sbcB* cells after RecBCD nuclease inactivation indicates that other, less efficient mechanisms may also be involved.

The kinetics of accumulation of pSF117 multimers after RecBCD nuclease inactivation in *sbcB* cells is biphasic (11). This property is even more prominent when synthesis of pOC81 linear multimers is followed under the same conditions. Linear multimer derivatives of pOC81 are detectable 60 min after temperature shift, and their proportion increases thereafter. The biphasic nature of the kinetics curves may be due to the functioning of more than one mode of initiation in this system. A relatively inefficient process, such as initiation from random nicks, may account for the first phase of linear multimer synthesis. Double-strand ends or 3′ OH single-strand ends which are produced during the primary process may then serve as recombinogenic elements to initiate the second phase of linear multimer synthesis.

The difference in the kinetics of linear multimer synthesis between pOC81 and pSB5 or pSF117 after RecBCD nuclease inactivation in *sbcB* mutants may be due either to the low copy number of OriC derivatives or to the absence of genetic elements which are involved in linear multimer synthesis of pOC81. The observation that insertion of an n′ recognition sequence (39) into pOC81 greatly enhances the rate of multimer synthesis by this plasmid (A. Cohen, unpublished data) is consistent with the second possibility.

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