Nucleic Acids Research, Vol. 20, No. 7 1733–1737

DnaG-dependent priming signals can substitute for the two essential DNA initiation signals in *oriV* of the broad host-range plasmid RSF1010

Yoichi Honda, Toshiaki Nakamura, Kastunori Tanaka, Akihisa Higashi, Hiroshi Sakai, Tohru Komano* and Michael Bagdasarian[1]
Laboratory of Biochemistry, Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan and [1]Michigan State University and Michigan Biotechnology Institute, Lansing, MI 48909, USA

Received November 11, 1991; Revised and Accepted March 10, 1992

ABSTRACT

Broad host-range plasmid RSF1010 contains in the *oriV* region two DNA initiation signals, *ssiA*(RSF1010) and *ssiB*(RSF1010), which are essential for plasmid replication. Each of *ssiA* and *ssiB* could be substituted functionally by either of the two G4-type (DnaG-dependent) priming signals, the ori_c of bacteriophage G4 and an *ssi* signal from plasmid pSY343 (an R1 plasmid derivative). Functions of the chimeric *oriV*s of RSF1010 thus constructed were dependent on the RSF1010-specific replication proteins, RepA, RepB′ and RepC. When both of *ssiA* and *ssiB* were replaced by the G4-type *ssi* signals, functions of the chimeric *oriV*s were no longer dependent on RepB′ (RSF1010-specific DNA primase). The replication activities of the chimeric *oriV*s of RSF1010 were not influenced markedly by the type of heterologous priming signals they contained. It is conceivable that DNA replication of RSF1010 does not need the priming mechanism for lagging strand synthesis and proceeds by the strand displacement mechanism.

INTRODUCTION

Nucleotide sequences, present on each strand of the template DNA, that direct introduction of the priming enzymes and initiation of DNA synthesis have been isolated from many bacterial plasmids and are called single strand DNA initiation (*ssi*) signals. The priming mechanisms at the *ssi* signals of the plasmids include the primosome-dependent type (φX-type) (1, 2, 3), the DnaG-dependent type (G4-type) (4), the ABC-primosome-dependent type (5) and the plasmid-specific type such as that of the broad host-range plasmid RSF1010 (6, 7, 8). Another broad host-range plasmid RK2 also possesses at least two specific *ssi* signals in the *oriT* region (9). Although some of these *ssi* signals are dispensable for the plasmid replication *in vivo*, they are essential to maintain wild-type plasmid copy numbers (4, 10). Furthermore, *in vitro* studies have shown that in plasmids, such as pBR322 and R1, *ssi* signals are actually essential to initiate DNA replication (4, 11, 12). Judging by the mode of function and the genomic location of *ssi* signals in various plasmids, it is conceivable that some *ssi* signals should contribute to the specificity of plasmid-directed initiation events and to establishment of the replication forks (5, 13, 14).

RSF1010 is an IncQ plasmid, the DNA of which is 8684 base pairs (bp) in length. It has a remarkable ability for DNA replication in a wide variety of Gram-negative bacteria (8, 15). In *E. coli*, the replication of RSF1010 does not require the function of the genes *dnaA*, *B*, *C*, *G*, *T*, and *rpoB* (7, 16, 17, 18). Instead, three plasmid-specified proteins, RepA, RepB′, and RepC, exhibit RSF1010-specific helicase, primase, and initiator protein activities, respectively, and are essential to the replication of this plasmid (7, 19). *E. coli* DNA gyrase, SSB (single strand DNA binding protein) and the product of *dnaZ* (γ subunit of DNA polymarase III holoenzyme) are also required for the plasmid replication (19, 20). In the *oriV* region, three distinct *cis*-acting elements are essential to the plasmid replication (13). These include the *inc* repeats and the two essential DNA priming signals, *ssiA* and *ssiB*, which are located on the *l* and *r* strands, respectively (21). The *inc* repeats, 20-nucleotide tracts repeated three and a half times, are the binding sites for the RepC initiator protein. The two *ssi* signals consist of highly conserved 40-nucleotide sequences and exclusively recognized by the RepB′ primase (8, 21). When cloned in single strand phage vectors, these nucleotide sequences can direct priming of the complementary DNA strand synthesis in the presence of RepB′ protein *in vivo* (6) and *in vitro* (7). Moreover, the primosome assembly sites from bacteriophage φX174 or plasmid pACYC184 are able to substitute functionally for both of *ssiA* and *ssiB* in the replication of RSF1010 (13). Function of the chimeric $oriV_{RSF1010}$ containing two primosome assembly sites in place of *ssiA* and *ssiB* is no longer dependent on RepB′ but still dependent on RepA and RepC. It has been postulated that RepB′ primase actually directs the RSF1010-specific priming reaction after completion of the prepriming process that is dependent on RepA and RepC.

* To whom correspondence should be addressed

In this report we show that both *ssiA* and *ssiB* of RSF1010 may be substituted *in vivo* by two distinct G4-type *ssi* signals; one is the origin for complementary strand synthesis (ori_c) of bacteriophage G4 and the other is the *ssi* signal from plasmid pSY343 (a run-away plasmid derived from R1 (22)) which corresponds to the *ssi* signal directing the leading strand synthesis in R1 plasmid replication. These G4-type *ssi* signals require only *E. coli* DnaG primase without introducing the primosome and direct only the initiation of leading strand synthesis (4, 23, 24). The replication mechanism of RSF1010 DNA replication will also be discussed.

MATERIALS AND METHODS

Bacterial strains and plasmids

E. coli JM109 was used as a host bacterium. Plasmid pYH101VS (13), the mini-RSF1010 plasmid, contains the coding region of β-lactamase from pBR322 and a 444-bp *oriV* segment from RSF1010 [nucleotides 2335–2778] (8). pYH101VS was inserted into the multicloning sites of pHSG399, a chroramphenicol-resistance derivative of pUC19, to produce pHSG399/YH101VS (13). This recombinant plasmid was used to construct chimeric $oriV_{RSF1010}$ with heterologous *ssi* signals. The G4-type *ssi* signal of plasmid pSY343, *ssiA*(pSY343), was previously cloned as a 170-bp fragment into the *Sma*I site of multicloning sites in M13Δ*lac*184 that was an ori_c defective derivative of M13 phage containing *lacZ'* of M13mp18 (25). The ori_c segment of bacteriophage G4 [nucleotides 3798–4070] (26) was also inserted into the *Sma*I site of M13Δ*lac*184. The DNA fragments containing these G4-type *ssi* signals were excised from RF DNA of relevant recombinant M13Δ*lac*184 phages upon cleavage with *Sac*I, *Xba*I or *Hin*cII. Then they were inserted into the $oriV_{RSF1010}$ region of pHSG399/YH101VS, from which *ssiA* or *ssiB* had been removed by cleavage with *Sac*I plus *Xba*I or *Sac*I plus *Eco*RV, respectively. Miniplasmid DNA segments containing the chimeric $oriV_{RSF1010}$ were cut out from the vector plasmid by *Eco*RI cleavage, and self-circularized by T4 DNA ligase followed by transformation into *E. coli* cells. Generation of ampicillin (Amp) resistant transformants was a primary criterion for the functionl $oriV_{RSF1010}$. Helper plasmids pMMB2 (ColD-based recombinant plasmid carrying *repA, repB'* and *repC* of RSF1010) and its deletion derivatives pMMB2Δ5, pMMB2Δ23, pMMB2ΔAE and pMMB2ΔSS have been described (6, 19).

DNA manipulation

Restriction endonucleases and DNA-modifying enzymes were from Takara Shuzo (Kyoto) and New England Biolabs, Inc. Transformation of plasmid DNA into *E. coli* cells were performed by the method of Chung *et al* (27). Concentrations of antibiotics in selective media were: Amp, 50 μg/ml and kanamycin 100 μg/ml.

RESULTS

Replication activities of chimeric origins with G4-type *ssi* signals

Replication activities of the miniplasmid pYH101VS and its derivatives with heterologous *ssi* signals were examined by transformation into JM109 harboring pMMB2 as a helper plasmid (Fig. 1). This plasmid supplies the RepA, RepB' and RepC proteins, essential for the DNA replication starting from

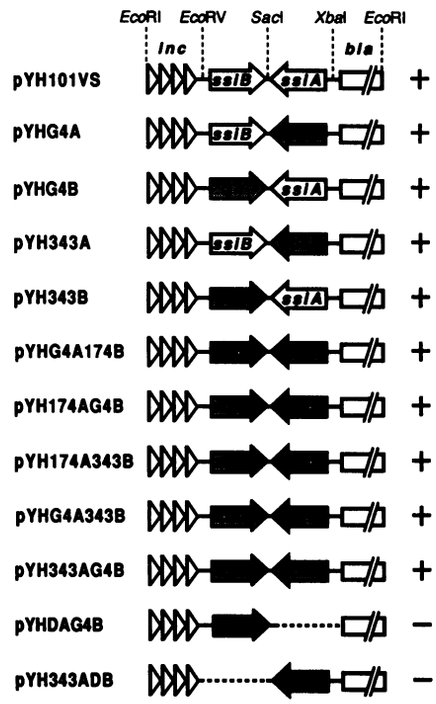

Fig. 1. Chimeric origins constructed from the mini-RSF1010 plasmid pYH101VS and their abilities to replicate in *E. coli* in the presence of RSF1010-specific Rep functions. Schematic representations of the miniplasmid derivatives were drawn as linearized at the unique *Eco*RI site. Restriction sites used to construct the miniplasmid derivatives are indicated. Open triangles indicate the *inc* repeats. Arrows indicate the locations and the priming orientations of the *ssi* signals: open arrows, the original RSF1010-specific *ssi* signals; shadowed arrows, the G4-type *ssi* signals from bacteriophage G4 or plasmid pSY343 (an R1 plasmid derivative); solid arrows, the primosome assembly site from bacteriophage φX174. Broken lines indicate the deleted areas. Open boxes indicate the coding region of the β-lactamase from pBR322. The ability (+) or inability (−) to produce stable transformants of JM109[pMMB2] by each miniplasmid derivative is indicated.

$oriV_{RSF1010}$. Miniplasmid derivatives pYHG4A, pYHG4B, pYH343A and pYH343B, which contained a G4-type signal from bacteriophage G4 or plasmid pSY343 instead of *ssiA* or *ssiB*, had abilities to replicate in this biochemical background. Moreover, miniplasmid derivatives pYHG4A174B, pYH174AG4B and pYH174A343B, in which one of the *ssiA* and *ssiB* sequences was replaced by a G4-type *ssi* signal and the other by the primosome assembly site from bacteriophage φX174, also could replicate. In addition, miniplasmid derivatives pYHG4A343B and pYH343AG4B, in which both of *ssiA* and *ssiB* were replaced by the G4-type *ssi* signals, could replicate as well. These findings indicate that not only the primosome assembly site but also G4-type *ssi* signals can substitute for the function of both *ssiA* and *ssiB* signals in the $oriV_{RSF1010}$. However, the miniplasmid derivatives containing a G4-type *ssi* signal on one complementary DNA strand but no *ssi* signal on the other (pYH343ADB and pYHDAG4B) gave no ampicillin-resistant transformants.

Requirements of RSF1010-specified Rep functions for the replication of the miniplasmid derivatives with heterologous *ssi* signals

In order to analyze the requirements of the RSF1010 Rep functions for the replication of the chimeric origins, DNAs of the miniplasmid derivatives with *ssi* substitutions were

Table 1. Requirements of RSF1010-specific Rep functions for the replication of miniplasmid derivatives.

miniplasmid derivative	Number of transformants with the helper plasmid pMMB2 (*repAB'C*)	pMMB2Δ5 (*repC*)	pMMB2Δ67 (*repAC*)	pMMB2ΔAE (*repB'*)
pYH101VS	6.5×10^5	nd	nd	nd
pYHG4A	2.3×10^5	nd	nd	nd
pYHG4B	3.2×10^5	nd	nd	nd
pYH343A	1.4×10^5	nd	nd	nd
pYH343B	1.9×10^5	nd	nd	nd
pYHG4A174B	1.3×10^5	nd	1.1×10^5	nd
pYH174AG4B	1.4×10^5	nd	1.7×10^5	nd
pYH174A343B	3.6×10^4	nd	2.9×10^4	nd
pYHG4A343B	8.3×10^4	nd	5.7×10^4	nd
pYH343AG4B	1.2×10^5	nd	1.3×10^5	nd

1.875 ng of self-ligated DNA fragments of the miniplasmid derivatives (see MATERIALS AND METHODS) were used to transform JM109 harboring pMMB2 or its deletion derivatives (13). Numbers of transformants per μg DNA are shown. Cases where no stable transformants were obtained are indicated by nd. Results are shown as mean values of two determinations.

Table 2. Relative copy numbers of *ssi*-substituted RSF1010 miniplasmid derivatives

miniplasmid derivative	*ssi* site A	*ssi* site B	Amp LD_{50}, μg/ml	Relative Copy number, %
pYH101VS	+	+	794.3	100
pYHG4A	G4	+	631.0	79.4
pYHG4B	+	G4	691.8	87.1
pYH343A	pSY	+	638.3	80.4
pYH343B	+	pSY	288.4	36.3
pYHG4A174B	G4	φX	645.7	81.3
pYH174AG4B	φX	G4	302.0	38.0
pYH174A343B	φX	pSY	257.0	32.4
pYHG4A343B	G4	pSY	354.8	44.7
pYH343AG4B	pSY	G4	638.3	80.4

Relative copy numbers were estimated by single-cell Amp^R levels. The *ssi* signals contained in the miniplasmid derivatives are indicated as follows: +, original RSF1010-specific *ssi* signal; G4, the G4-type *ssi* signal of bacteriophage G4; pSY, the G4-type *ssi* signal from pSY343 (an R1 plasmid derivative); φX, the primosome assembly site from φX174. Amp LD_{50} was determined by the method of Nordström *et al.* (28). Results are shown as mean values of two determinations with independent clones.

transformed into JM109 harboring the deletion derivatives of pMMB2 (Table 1). When pMMB2ΔSS containing no *rep* region of RSF1010 was supplied as a coresident helper plasmid, none of the miniplasmid derivatives could replicate (data not shown). Nor could they replicate in the presence of pMMB2Δ5 (*repC*$^+$, *repB'*$^-$, *repA*$^-$) or pMMB2ΔAE (*repB'*$^+$, *repC*$^-$, *repA*$^-$). However, the miniplasmid derivatives, in which both *ssiA* and *ssiB* were replaced by heterologous signals, could replicate in the absence of RepB' primase when RepA and RepC were supplied (pMMB2Δ67 as a coresident helper plasmid). On the other hand, miniplasmid derivatives pYHG4A, pYHG4B, pYH343A, pYH343B, in which only one of the *ssi* signals was replaced by a G4-type signal, could not replicate in the presence of this helper plasmid. These results are in good agreement with the previous report showing that the RepB' protein was not required for the replication of pYH174A184B in which both *ssiA* and *ssiB* are replaced by the primosome assembly sites, but required for the function of those chimeric origins in which only one *ssi* signal was substituted (13). These findings supported the hypothesis that, in the initiation stage of RSF1010 DNA replication, RepB'-dependent priming reaction is separated from the prepriming process dependent on both of RepC and RepA proteins which act as an initiator and a DNA helicase, respectively (13, 20).

Copy number estimation of the miniplasmid derivatives

Relative copy numbers of the miniplasmid derivatives with various *ssi* substitutions were determined by the single cell Amp resistance method (28) in JM109 harboring pMMB2 (Table 2). Relative copy numbers of miniplasmid derivatives pYHG4A, pYHG4B, pYH343A, pYHG4A174B and pYH343AG4B were approximately 80 per cent of that of pYH101VS. The function of one or both of the *ssi* signals in $oriV_{RSF1010}$ can be substituted by the G4-type *ssi* signals without serious decrease in the replication activities of the chimeric origins. However, relative copy numbers of the other miniplasmid derivatives appeared to be about half of those of the miniplasmids described above. The relative copy number of the miniplasmid derivative pYH174A, which contained the primosome assembly site from φX174 in place of *ssiA*, also appeared to be about half of that of pYH101VS (13). It is notable that the chimeric origins of the miniplasmid derivatives with reduced replicative activities always contained substitutions of *ssiA* by the primosome assembly site from φX174 or of *ssiB* by the G4-type *ssi* signal from pSY343. This is true without exception. It seems that these decrease in replication activities were caused not by the functional difference between *ssiA* and *ssiB* but by some structual changes in the chimeric origins, because substitutions of *ssiA* by the primosome assembly

site from pACYC184 (13) or of *ssiB* by the G4-type *ssi* signal from G4 did not cause the decrease in the copy numbers of the miniplasmid derivatives. In $oriV_{RSF1010}$, *ssiA* and *ssiB* sequences are involved in a large inverted repeat structure [nucleotides 2589–2739] and this region contains typical DNA bending motif extending over the 90 bp stretch between the *ssi* signals. RepC-induced DNA looping structures which include this large inverted repeat region have been observed *in vitro* (7). Each substitution of the *ssi* signals should make some changes in these structures and these structual changes may affect the replication activities of the chimeric origins. However, we can not rule out the possibility that the paticular *ssi* substitutions described above might affect the expression of the closely flanking *bla* gene.

DISCUSSION

In *E. coli*, priming enzymes are introduced into origins of DNA replication through various mechanisms that depend on the specific nucleotide sequences. The *ssi* signals direct entry of the primase molecules depending on the specific nucleotide sequences in single strand DNA templates (5, 6, 7, 14). Specific nucleotide sequences in double strand DNAs, such as the dnaA box in *oriC* (29) and the direct repeats of *ori*λ (30), introduce DnaG primase efficiently in consequence of a series of protein-DNA and protein-protein interactions. Furthermore, sequence non-specific mechanisms (such as the DnaT-dependent mechanism and so on (4, 10)) which function with single-stranded DNA have also been reported. However, these sequence non-specific priming mechanisms are not efficient enough to meet the requirements for normal replication *in vivo* (10, 12). Plasmids or phages parasitic to *E. coli* should have their own efficient mechanisms to ensure introduction of the priming enzymes and establishment of the replication forks. In RSF1010, the *oriV* region contains no priming signals dependent on *E. coli* factors, and *in vitro* experiments have shown that *E. coli* replication proteins DnaA, B, C, G and T are not required for DNA replication of RSF1010 (20, 31). Instead, the RSF1010-specific priming apparatus involves RepB′ primase and its specific recognition sequences, *ssiA* and *ssiB*, in the template strands (7, 13). The function of the two RSF1010-specific *ssi* signals may be substituted by the primosome assembly sites (13). However, it is not clear whether these RSF1010-specific *ssi* signals should serve as the unique primer sites or the entry sites for the possible RSF1010-specific 'primosomes' involving RepB′ primase and the DNA helicase such as RepA protein. Such replicon-specific 'primosomes' have actually been reported for bacteriophage T4 (32) and T7 (33).

In the present study, we have shown that the two distinct G4-type priming signals from bacteriophage G4 and plasmid pSY343 can also substitute for the function of the two RSF1010-specific *ssi* signals. These G4-type *ssi* signals direct the priming of DNA depending on DnaG primase alone and have no abilities to direct the lagging strand synthesis (4, 24). It is conceivable that, in cooperation with RepB′ primase, *ssiA* and *ssiB* provide the primer molecules exclusively for the leading strand synthesis, and that DNA replication of RSF1010 does not require a primary system for lagging strand synthesis. Recently, with the electron microscopic analysis, Scherzinger *et al.* have observed the replicative intermediates in the *in vitro* cell-free replication system of RSF1010 (20), suggesting that two displacement events may begin at or near the *ssi* sites. The two displacement strands seemed to start in a synchronous or an asynchronous manner and expand toward each other. Based on these results, it is conceivable that, although sequence non-specific priming mechanisms are functional in *E. coli*, the RSF1010 DNA replication does not involve primarily the discontinuous lagging strand synthesis and replication forks proceed mainly by the strand displacement mechanism producing D-loop intermediates.

The $oriV_{RSF1010}$ involves various specific structures, such as large inverted repeat and DNA bending motif, which should have some roles in the initiation of DNA replication. Moreover, superhelicity of the plasmid DNA is essential for the *in vitro* RSF1010 DNA replication (20). Wada *et al.* have shown that HU protein, the major histone-like protein of *E. coli* which has the abilities to induce DNA looping or bending (34), should be required for the intracellular maintenance of RSF1010 (35). Specific high-ordered molecular structure of the $oriV_{RSF1010}$ may play significant roles in the initial stage of RSF1010 DNA replication.

ACKNOWLEDGEMENT

This work was supported in part by grants from the Ministry of Education, Science and Cultutre, Japan.

REFERENCES

1. Nomura, N. and Ray, D. S. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 6566–6570.
2. Nomura, N., Low, R. L. and Ray, D. S. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 3153–3157.
3. Zipursky, S. N. and Marians, K. J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6111–6115.
4. Masai, H. and Arai, K. (1989) *J. Biol. Chem.*, **264**, 8082–8090.
5. Masai, H., Nomura, N., Kubota, Y. and Arai, K.-i. (1990) *J. Biol. Chem.*, **265**, 15124–15133.
6. Honda, Y., Sakai, H., Komano, T. and Bagdasarian, M. (1989) *Gene*, **80**, 155–159.
7. Haring, V. and Scherzinger, E. (1989) In Thomas, C. and Franklin, F. C. H. (eds.), Promiscuous Plasmids of Gram-negative Bacteria, Academic Press, London, pp. 95–124.
8. Scholz, P., Haring, V., Wittman-Liebold, B., Ashman, K., Bagdasarian, M. and Scherzinger, E. (1989) *Gene*, **75**, 271–281.
9. Yakobson, E., Cornelia, D., Hirata, K. and Guiney, D. G. (1990) *Plasmid*, **23**, 80–84.
10. van der Ende, A., Teertstra, R. and Weisbeek, P. J. (1983) *J. Mol. Biol.*, **167**, 751–756.
11. Minden, J. S. and Marians, K. J. (1985) *J. Biol. Chem.*, **260**, 9316–9325.
12. Masai, H. and Arai, K. (1988) *J. Biol. Chem.*, **263**, 15016–15023.
13. Honda, Y., Sakai, H., Hiasa, H., Tanaka, K., Komano, T. and Bagdsarian, M. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 179–183.
14. Masai, H., Nomura, N. and Arai, K.-i. (1990) *J. Biol. Chem.*, **265**, 15134–15144.
15. Grinter, N. J. and Barth, P. T. (1976) *J. Bacteriol.*, **128**, 394–400.
16. de Graff, J., Crosa, J. H., Heffron, F. and Falkow, S. (1978) *J. Bacteriol.*, **134**, 1117–1122.
17. Scholz, P., Haring, V., Scherzinger, E., Lurz, R., Bagdasarian, M. M., Schuter, H. and Bagdasarian, M. (1985) In Helinski, D. R., Cohen, S. N., Clewell, D. B., Jackson, D. A. and Hollaender, A. (eds.), Plasmids in Bacteria, Plenum, New York, pp. 243–259.
18. Frey, J. and Bagdasarian, M. (1989) In Thomas, C. and Franklin, F. C. H. (eds.), Promiscuous Plasmids of Gram-negative Bacteria, Academic Press, London, pp. 79–94.
19. Scherzinger, E., Bagdasarian, M. M., Scholz, P., Lurz, R., Rückert, B. and Bagdasarian, M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 654–658.
20. Scherzinger, E., Haring, V., Lurz, R. and Otto, S. (1991) *Nucl. Acids Res.*, **19**, 1203–1211.
21. Honda, Y., Sakai, H. and Komano, T. (1988) *Gene*, **68**, 221–228.
22. Uhlin, B. E., Molin, S., Gustafsson, P. and Nordström, K. (1979) *Gene*, **6**, 91–106.
23. Bouché, J., Rowen, L. and Kornberg, A. (1978) *J. Biol. Chem.*, **253**, 765–769.
24. Sims, J., Capon, D. and Dressler, D. (1979) *J. Biol. Chem.*, **254**, 12615–12628.

25. Bahk, J., Kioka, N., Sakai, H. and Komano, T. (1988) *Plasmid,* **20**, 266–270.
26. Godson, G. N., Barrell, B. G., Staden, R. and Fidds, J. C. (1978) *Nature,* **276**, 236–247.
27. Chung, C. T., Niemela, S. L. and Miller, R. H. (1989) *Proc. Natl. Acad. Sci. USA,* **86**, 2172–2175.
28. Nordström, K., Molin, S. and Aagaard-Hansen, H. (1980) *Plasmid,* **4**, 332–349.
29. Funnell, B. E., Baker, T. A. and Kornberg, A. (1987) *J. Biol. Chem.*, **262**, 10327–10334.
30. Schnös, M., Zahn, K., Inman, R. B. and Blattner, F. R. (1988) *Cell,* **52**, 385–395.
31. Haring, V., Scholz, P., Scherzingaer, E., Frey, J., Derbyshire, K., Hatfull, G., Willetts, N. S. and Bagdasarian, M. (1985) *Proc. Natl. Acad. Sci. USA,* **82**, 6090–6094.
32. Selick, H. E., Barry, J., Cha, T., Munn, M., Nakanishi, M., Wong, M. L. and Alberts, B. M. (1987) In McMcken, R. and Kelly, T. J. (eds.), DNA replication and recombination, Alan R. Liss, Inc, New York, pp. 183–214.
33. Richardson, C. C., Beauchamp, B. B., Huber, H. E., Ikeda, R. A., Myers, J. A., Nakai, H., Rabkin, S. D., Tabor, S. and White, J. (1987) In McMacken, R. and Kelly, T. J. (eds.), DNA replication and recombination, Alan R. Liss, Inc, New York, pp. 151–171.
34. Drlica, K. and Rouviere-Yanif, J. (1987) *Microbiol. Rev.*, **51**, 301–319.
35. Wada, M., Kano, Y., Ogawa, T., Okazaki, T. and Imamoto, F. (1988) *J. Mol. Biol.*, **204**, 581–591.