Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7845–7849, December 1984
Genetics

Absence of RNase H allows replication of pBR322 in *Escherichia coli* mutants lacking DNA polymerase I

(*polA/sdrA/rnh*)

TOKIO KOGOMA

Departments of Biology and Cell Biology/Cancer Center, University of New Mexico, Albuquerque, NM 87131

Communicated by Evelyn M. Witkin, August 9, 1984

ABSTRACT ***rnh* (formerly termed *sdrA*) mutants of *Escherichia coli* K-12, capable of continuous DNA replication in the absence of protein synthesis (stable DNA replication), are devoid of ribonuclease H (RNase H, EC 3.1.26.4) activity. Plasmid pBR322 was found to replicate in *rnh* mutants in the absence of DNA polymerase I, the *polA* gene product, which is normally required for replication of this plasmid. The plasmid copy number in *polA rnh* double mutants was as high as in the wild-type strains. When a chimeric construct between pBR322 and pSC101 was introduced into a *polA rnh* double mutant, the replication of the plasmid via the pBR322 replicon was inhibited if the plasmid also carried an rnh^+ gene or if the host harbored an F′ plasmid carrying an rnh^+ gene. Thus, DNA polymerase I-independent replication of pBR322 requires the absence of RNase H activity. This alternative mechanism requiring neither DNA polymerase I nor RNase H appears to involve a transcriptional event in the region of the normal origin of replication.**

ColE1 is an amplifiable plasmid that continues to replicate in the absence of *de novo* protein synthesis (1). Initiation of the replication of ColE1 has been shown, *in vivo* (2) and *in vitro* (3, 4), to require active DNA polymerase I. The replication involves an RNA primer (RNA II), the product of a specific transcription initiated at a promoter (P*ori*) by DNA-dependent RNA polymerase. RNase H (EC 3.1.26.4), encoded by the rnh^+ gene (5), degrades RNA specifically in DNA·RNA hybrid molecules and is involved in the processing of a preprimer RNA at the origin (*ori*) of plasmid replication *in vitro* (6). The processed RNA molecule is used as primer by DNA polymerase I. It has been shown that, *in vitro*, in the absence of RNase H, plasmid replication is initiated at sites other than the ColE1 *ori* site (7).

sdrA mutants of *Escherichia coli* can initiate rounds of chromosome replication without the participation of the normal replication origin (*oriC*) (8). These *sdrA* mutants were isolated by virtue of their capacity for $recA^+$-dependent DNA replication in the absence of *de novo* protein synthesis (stable DNA replication) (9, 10). Since initiation in *sdrA* mutants can occur not only in the absence of the *oriC* sequence but also in the complete absence of the $dnaA^+$ gene product, an initiator protein (8), it has been postulated that initiation in *sdrA* mutants represents an alternative initiation pathway distinct from the normal $oriC^+$ $dnaA^+$-dependent initiation mechanism (8, 11). Several sites or regions of the *E. coli* chromosome have been detected from which initiation in *sdrA* mutants occurs in the absence of *oriC* (12).

It has recently been shown that *sdrA* mutants are devoid of RNase H activity (13). The demonstration that a small fragment of the *E. coli* genome, which contains no complete gene other than the rnh^+ gene (14), complements all the phenotypes of *sdrA* mutants has led to the conclusion that *sdrA* mutations are allelic to *rnh* mutations (13).

Plasmid pBR322, which was derived from pMB1, a ColE1-type plasmid, requires DNA polymerase I for the initiation of replication, as it does not replicate in *polA* mutants (15). In this communication, I report that pBR322 can replicate in *rnh* (*sdrA*) mutants despite the absence of DNA polymerase I activity. A preliminary account of this work has been reported (16).

MATERIALS AND METHODS

E. coli strain AQ666 is an *rnh-224* (formerly *sdrA224*) pro^+ derivative of AQ634 (17) [F^- *argH ilv metB his-29 trpA9605 pro-2 thyA deoB* (or *deoC*)]. AQ1523 and AQ1525 are *polA12* derivatives of AQ634 and AQ666, respectively. AQ2178 and AQ2190 are *polA1* derivatives of AQ634 and AQ666, respectively. The *polA12* and *polA1* mutations were introduced by P1-mediated transduction from CM5389 and CM5280 (18), respectively, by virtue of linkage to a Tn*10* insertion (*zig*::Tn*10*). The $PolA^-$ phenotype was determined by sensitivity to methyl methanesulfonate (19). AQ634 and AQ2190 were transformed to ampicillin-resistant (Ap^r) with pBR322, resulting in AQ990 and AQ2199, respectively. The strain AQ2158, used to determine the requirement for DNA polymerase III in plasmid replication in the absence of DNA polymerase I, was constructed by transducing *rnh-224* into H10265 (*polA1* $polB^-$ *dnaE1026*) (ref. 20) and transforming a resultant strain (AQ1351) to Ap^r with pTKQ51 (Fig. 1). Since *rnh* mutants are sensitive to broth (17), transformation frequencies were determined using M9 glucose plates supplemented with Casamino acids. The construction of plasmids used in this work is described in Fig. 1. All media, chemicals, and growth conditions used were previously described (9). Restriction endonucleases and T4 DNA ligase were obtained from Bethesda Research Laboratories. Protein and β-galactosidase assays were done according to refs. 25 and 26, respectively.

Measurement of replication of plasmid and *E. coli* DNA (Table 3) was performed as follows. Cultures of AQ990 and AQ2199 were labeled, at 37°C in medium containing [^{14}C]-thymine (2.5 μCi/8 μg per ml; 1 Ci = 37 GBq), for about 2 doublings to a cell density of 3×10^8 cells per ml. The cells were collected on filters, washed with warm M9 medium, and suspended in warm fresh medium. Each culture was then split into three 1.5-ml parts. One portion (control) immediately received [^{3}H]thymine (30 μCi/8 μg per ml) whereas [^{3}H]thymine was added to the second and third portions only after they were incubated for 60 min in the presence of chloramphenicol (CAP, 150 μg/ml) and rifampicin (Rif, 250 μg/ml), respectively. All cells were labeled with [^{3}H]thymine for 60 min. The ^{3}H and ^{14}C in covalently closed circular

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ap^r, ampicillin-resistant; Ap^s, ampicillin-sensitive; CAP, chloramphenicol; Rif, rifampicin; bp, base pair(s).

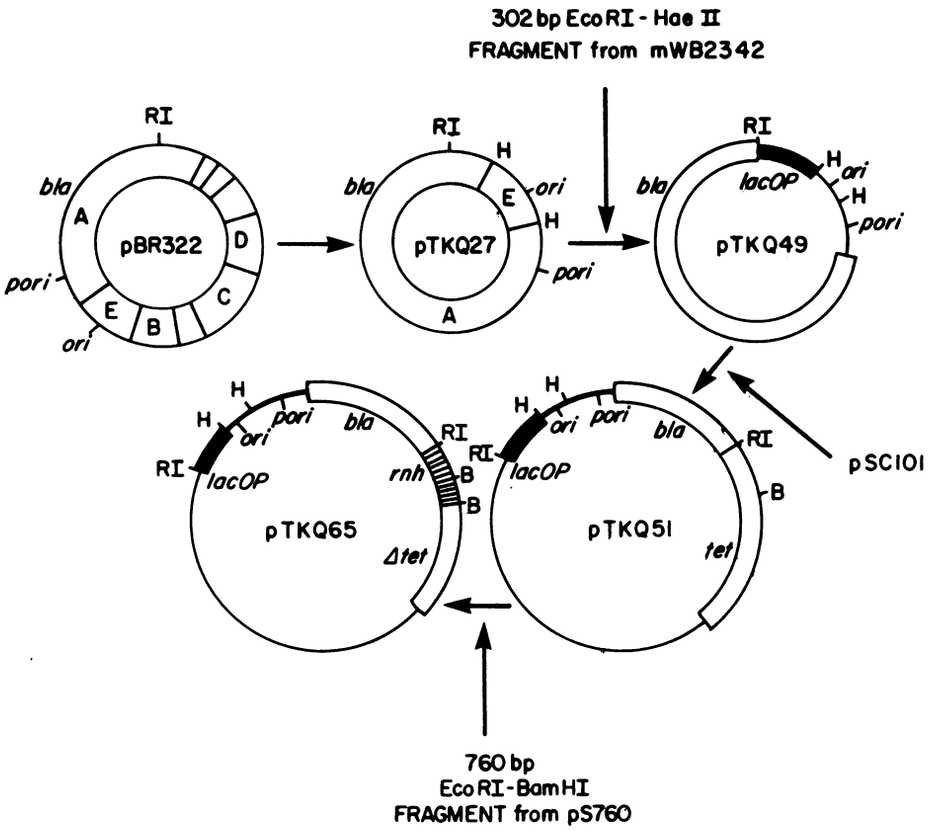

FIG. 1. Construction of plasmids. pBR322 was digested to completion with restriction endonuclease *Hae* II and ligated with T4 DNA ligase. The resulting plasmid DNA was used to transform an Ap^s *rnh*$^-$ *polA12* strain to Ap^r at 42°C. pTKQ27 was one of the smallest among the plasmids from these Ap^r transformants. It consisted of *Hae* II fragments A and E (21) in the same orientation as found in pBR322. The identification and orientation of the fragments were determined by restriction endonuclease analysis (21). In the next step, the 236-bp *Eco*RI/*Hae* II fragment of pTKQ27 was replaced with the the 302-bp *Eco*RI/*Hae* II fragment (*lacOP*) of mWB2342, which contained the *lac* promoter and operator sequences (22). The resulting plasmid, pTKQ49, was subsequently joined to pSC101 (23) at the *Eco*RI sites, yielding pTKQ51. Finally, the 375-bp *Eco*RI/*Bam*HI fragment from the *tet* region of pTKQ51 was substituted with the 760-bp *Eco*RI/*Bam*HI fragment carrying the *rnh*$^+$ gene from pSK760 (14) to yield pTKQ65. Restriction sites: RI, *Eco*RI; H, *Hae* II; B, *Bam*HI. P*ori*, promoter for the synthesis of the pre-primer RNA; *ori*, the origin of normal replication; *bla*, β-lactamase gene (Ap^r); *tet*, tetracycline-resistance gene (Tc^r). The designation of *Hae* II fragments of pBR322 is according to ref. 21. In a previous paper (16), the assignment of the fragments according to ref. 24 was used. Thus, the fragment E in this figure is identical to the previous fragment D (16).

plasmid DNA and linear *E. coli* DNA fractions were determined (27).

RESULTS

***polA rnh* Double Mutants Can Be Transformed with pBR322.** Plasmid pBR322 transforms ampicillin-sensitive (Ap^s) cells to Ap^r when the plasmid is allowed to replicate in the cells. A *polA1* mutant (AQ2178) could not be transformed by pBR322 (Table 1). However, after introduction of the *rnh-224* mutation, the *polA1* mutant cells (AQ2190) could be efficiently transformed to Ap^r, albeit at a slightly lower frequency than the wild-type cells (AQ634) (Table 1). *polA12* is a missense mutation giving rise to a temperature-sensitive DNA polymerase I (28). The results summarized in Table 1 also show that *polA12 rnh*$^+$ cells (AQ1523) could not be transformed by pBR322 at 42°C whereas *polA12 rnh-224* cells (AQ1525) could. pSC138, containing a mini-F replicon (29) that does not require DNA polymerase I activity for replication (30), transformed the *polA12* cells (at 37°C) with similar frequencies regardless of the presence or absence of the *rnh* mutation (data not shown). Therefore, the inability of *polA*$^-$ *rnh*$^+$ cells to be transformed with pBR322 is due to the defective DNA polymerase I, and the *rnh* mutation alleviates this deficiency for plasmid replication.

A Minimum Sequence Required for DNA Polymerase I-In-

Table 1. Transformation frequencies of *polA* mutants with Ap^r plasmids

Strain	Relevant genotype	Temperature, °C	Ap^r transformants/ng of DNA		
			pBR322	pTKQ27	pTKQ51
AQ634	*rnh*$^+$ *polA*$^+$	37	130.0	183.0	13.5
AQ2178	*rnh*$^+$ *polA1*	37	$<10^{-2}$	$<10^{-2}$	14.9
AQ2190	*rnh-224 polA1*	37	17.0	34.9	4.49
AQ1523	*rnh*$^+$ *polA12*	30	0.121	0.031	ND
		42	$<10^{-3}$	$<10^{-3}$	ND
AQ1525	*rnh-224 polA12*	30	7.20	2.06	ND
		42	9.60	2.25	ND

ND, Not done.

dependent Replication Includes the Normal Origin of Replication. To determine a minimun nucleotide sequence required for replication in *polA*$^-$ *rnh*$^-$ conditions, pBR322 DNA was digested with *Hae* II, ligated with T4 DNA ligase, and subsequently used to transform Ap^s *polA12 rnh-224* cells to Ap^r at 42°C. The smallest plasmid obtained that still transformed *polA*$^-$ *rnh*$^-$ cells was pTKQ27, consisting of the A and E *Hae* II fragments (21) (Fig. 1 and Table 1). Repeated attempts to find a plasmid with the E fragment in the reversed orientation, after digestion of pTKQ27 to completion with *Hae* II and subsequent ligation of the digest, failed. Among viable plasmids found was one containing two E fragments in tandem in the same orientation as in pBR322 (data not shown). These observations suggested that the sequence between P*ori* and *ori* at the junction of *Hae* II fragments A and E (21) cannot be disturbed without adversely affecting plasmid replication in *polA*$^-$ *rnh*$^-$ conditions. pTKQ27 was found to also replicate in *rnh*$^+$ *polA*$^+$ cells (Table 1).

Plasmid Replication in *rnh*$^-$ *polA*$^-$ Cells Occurs at a High Copy Number. A chimera between a high-copy-number (such as pBR322) and a low-copy-number plasmid (pSC101) replicates at a high copy number (31). Only when the replication from the origin of the high-copy-number plasmid is prevented does the chimera replicate as a low-copy-number plasmid (31). pTKQ51 is a chimeric construct between pTKQ27 and pSC101 and also carries a 302-base-pair (bp) DNA fragment containing the *E. coli lac* operator-promoter sequence (Fig. 1). pTKQ51 transformed *polA*$^-$ cells at similar frequencies in the presence or absence of *rnh* mutations (Table 1), because pSC101 does not require DNA polymerase I for replication (31). It was expected that the synthesis of β-galactosidase in *lac*$^+$ strains would be gratuitously induced when the chimeric plasmid pTKQ51 existed at a high copy number. This gratuitous induction results from sequestering of the *lac* repressor molecules by the *lac* operator sequence that exists at a high copy number. On the other hand, the *lac*$^+$ operon expression would be minimal when pTKQ51 existed at a low copy number. The results summarized in Table 2 show that introduction of pTKQ51 into *lac*$^+$ *polA*$^+$ strains in the presence or absence of *rnh* mutations resulted in a 100-fold increase in β-galactosidase activity. This indicates that the plasmid replicated at high copy numbers both in the presence and absence of RNase H (in *polA*$^+$), confirming the previously reported observation (32). On the other hand, in *polA*$^-$ *rnh*$^+$ cells harboring pTKQ51, the increase in *lac* expression was limited to only about 10-fold, suggesting that pTKQ51 was present at a low copy number, as expected for the plasmid being replicated from the pSC101 origin (Table 2). In contrast, *lac* expression in *polA*$^-$ *rnh*$^-$ cells harboring pTKQ51 was nearly as high as that in pTKQ51-carrying *polA*$^+$ cells. The results suggested that plasmid replicated at a high copy number under *polA*$^-$ *rnh*$^-$ conditions. This was confirmed by direct measurements of the copy number, which involved labeling of plasmid-containing cells with [^{3}H]thymine and subsequent separation of plasmid DNA from linear DNA by dye/CsCl equilibrium centrifugation (27). The results of such measurements indicated that *polA*$^-$ *rnh*$^-$ cells contained nearly as many copies of pBR322 or pTKQ27 as *polA*$^+$ *rnh*$^+$ cells did (data not shown).

Table 2. β-Galactosidase activity of strains carrying pTKQ51 or pTKQ65

Strain	Relevant genotype	Plasmid	β-Galactosidase, units/mg of protein
AQ1523	*rnh*$^+$ *polA*$^-$	None	28.1
AQ1525	*rnh*$^-$ *polA*$^-$	None	36.3
AQ634	*rnh*$^+$ *polA*$^+$	pTKQ51	3338.2
		pTKQ65	2870.5
AQ666	*rnh*$^-$ *polA*$^+$	pTKQ51	2925.0
		pTKQ65	1863.9
AQ2178	*rnh*$^+$ *polA*$^-$	pTKQ51	400.2
		pTKQ65	327.4
AQ2190	*rnh*$^-$ *polA*$^-$	pTKQ51	2351.5
		pTKQ65	339.6

Cells were grown at 37°C in M9 glycerol (0.1%) medium supplemented with Casamino acids (0.2%) and other growth requirements. When cell density reached 2–4 × 10^8 cells per ml, the cultures were rapidly chilled in ice water. Aliquots (0.5 ml) were shaken vigorously with a drop of toluene at 37°C for 30 min and assayed for β-galactosidase activity (26). Under these conditions, a culture of AQ634 that had been fully induced for β-galactosidase with isopropyl-β-D-thiogalactoside (2.5 × 10^{-4} M) gave 6200 units/mg of protein.

DNA Polymerase I-Independent Replication Requires Absence of RNase H. *rnh-224* mutants are devoid of RNase H activity (<0.05% of the wild-type activity) (13). It has been shown that plasmid pSK760 introduced into *rnh-224* mutants restores intracellular RNase H activity to a level 10-fold higher than that of *rnh*$^+$ cells (13). pSK760 is a pBR322 derivative that carries the entire gene for RNase H (*rnh*$^+$) in a 760-bp *Eco*RI/*Bam*HI segment of the *E. coli* chromosome (14). pTKQ65 was constructed by substituting the 375-bp *Eco*RI/*Bam*HI fragment in the *tet* region of pTKQ51 with the *rnh*$^+$ fragment from pSK760 (Fig. 1). When pTKQ65 was introduced into *polA*$^+$ *rnh*$^+$ cells (AQ634), the *lac* expression was slightly reduced from the level induced by pTKQ51 (Table 2). A similar result was obtained with *polA*$^+$ *rnh-224* cells (AQ666) (Table 2). These results are consistent with the observation that the presence of excess amounts of RNase H inhibits DNA polymerase I-dependent replication of ColE1 in an *in vitro* replication system (33) and suggests that overproduction of RNase H may be inhibitory to *polA*$^+$-dependent replication of ColE1-type plasmids *in vivo*.

When pTKQ65 was introduced into *polA*$^-$ *rnh*$^-$ cells (AQ2190), the *lac* expression was reduced to a level comparable to that of *polA*$^-$ *rnh*$^+$ cells (AQ2178) (Table 2). A similar degree of reduction in *lac* expression was also observed when F'128, carrying a single copy of *rnh*$^+$ (9, 17), was introduced into *rnh*$^-$ *polA*$^-$ cells harboring pTKQ51 (16). These results indicate that DNA polymerase I-independent plasmid replication is eliminated by the presence of RNase H activity.

DNA Polymerase I-Independent Replication Is Sensitive to Rif. *In vivo* replication of ColE1-type plasmids including pBR322 in *polA*$^+$ cells has been shown to be sensitive to Rif, an RNA synthesis inhibitor, although it continues in the presence of CAP (34). To determine whether *polA*$^+$-independent replication of pBR322 in the absence of RNase H is sensitive to Rif or CAP, incorporation of [^{3}H]thymine into plasmid DNA in the presence of Rif or CAP was examined by use of dye/CsCl equilibrium centrifugation (27). The results summarized in Table 3 show that replication of pBR322 in *polA*$^-$ *rnh*$^-$ cells occurred in the presence of CAP as well as it did in *polA*$^+$ *rnh*$^+$ cells. On the other hand, pBR322 replication in *polA*$^-$ *rnh*$^-$ conditions was severely inhibited with Rif. The degree of the inhibition in *polA*$^-$ *rnh*$^-$ cells with Rif was comparable to that in the *polA*$^+$ *rnh*$^+$ cells.

Requirement for DNA Polymerase III. Normal replication of ColE1-type plasmids requires active DNA polymerase III (35), encoded in part by the *polC* (*dnaE*) gene. An experiment very similar to the one described above (Table 3) was performed, using an *rnh*$^-$ *polA*$^-$ *polB*$^-$ *polC*^{ts} strain harboring pTKQ51 (see *Materials and Methods*), to determine the requirement for DNA polymerase III in *polA*$^+$-independent plasmid replication. Cells, prelabeled with [^{14}C]thymine at 30°C, were incubated with [^{3}H]thymine for 60 min at either 30°C or 42°C. DNA was extracted from the cells and analyzed for radioactivity in plasmid (covalently closed circular) and chromosomal (linear) DNA. The ^{3}H/^{14}C ratios in the plasmid DNA fraction and linear DNA fraction at 30°C were

Table 3. Effects of Rif or CAP on replication of plasmid and *E. coli* DNA

Strain	Relevant genotype	Treatment	$^3H/^{14}C$ (replication, %) Plasmid	*E. coli*
AQ990	*rnh*$^+$ *polA*$^+$ (pBR322)	Control	14.17 (100)	11.5 (100)
		CAP	4.34 (30.6)	1.76 (15.8)
		Rif	0.45 (3.1)	0.76 (6.9)
AQ2199	*rnh*$^-$ *polA*$^-$ (pBR322)	Control	3.25 (100)	4.57 (100)
		CAP	1.19 (36.6)	0.98 (21.4)
		Rif	0.06 (1.9)	0.16 (3.4)

Control AQ990 and AQ2199 cultures were labeled with [^{3}H]thymine for 60 min, during which cells were allowed to grow with doubling times of 57 and 60 min, respectively, while the other cultures were treated with antibiotics before and during labeling (see *Materials and Methods*). Therefore, in a strict sense, they are not controls. Accordingly, replication (%) in drug-treated cultures is underestimated by a factor of ≈2. Plasmid DNA and chromosomal *E. coli* DNA were separated (27) as covalently closed circular DNA and linear DNA, respectively.

3.46 and 4.79, respectively. On the other hand, at 42°C, the restrictive temperature for the temperature-sensitive DNA polymerase III, the ratios were 0.79 for plasmid DNA and 0.82 for chromosomal DNA. Thus, plasmid replication in the absence of DNA polymerase I is as temperature sensitive as *E. coli* DNA replication in the *polC*^{ts} mutant, indicating the requirement for DNA polymerase III.

DISCUSSION

Recently, Masukata and Tomizawa (36) isolated a number of point mutations of plasmid ColE1 that caused defective plasmid replication. One of those mutations (*pri5*) altered the RNase H cleavage site of the pre-primer RNA *in vitro*. The resulting altered primer RNA was very inefficient in priming of DNA synthesis by DNA polymerase I *in vitro*. Their results provided additional evidence for the involvement of RNase H in *in vitro* replication of ColE1 (36).

In contrast to the *in vitro* evidence, it was found that *rnh* mutations that abolished RNase H activity had no detectable effects on the *in vivo* replication of ColE1-type plasmids (ref. 32; see also Table 2). More recently, Naito *et al.* (37) reported that another *rnh* mutation (*herA*), which suppressed the replication defect of a ColE1 mutant (*cer6*), also supported replication of the wild-type plasmid. They postulated that ColE1 replication *in vivo* proceeds in the absence of RNase H activity.

This apparent paradox can perhaps be resolved in light of the results described in this report. It is probable that RNase H is required for *in vivo* formation of active primer RNA for DNA polymerase I. However, inactivation of RNase H by *rnh/sdrA/herA* mutations allows plasmid replication to proceed in an alternative manner that requires neither the primer-processing activity of RNase H nor DNA polymerase I activity. Plasmid mutants that produce a defective RNA primer due to lack of or altered processing by RNase H replicate in *rnh*$^-$ cells because the alternative initiation mechanism bypasses the requirement for DNA polymerase I, which is dependent on a precise primer structure (36).

The mechanism by which plasmid replication occurs in the absence of DNA polymerase I and RNase H activity is not understood. In addition to the clear distinction of *polA*$^+$ independence, plasmid replication in *rnh*$^-$ conditions also differs from normal replication in that it generates concatemeric forms—i.e., covalently closed circular oligomers consisting of several plasmid units joined in a head-to-tail fashion (16). This concatemer formation is *recA*$^+$ dependent (16).

Despite these differences, the requirements of this alternative replication pathway resemble those of the normal *polA*$^+$-dependent pathway. First, the results of experiments to determine a minimum sequence required for plasmid replication in *polA*$^-$ *rnh*$^-$ conditions (Fig. 1) strongly suggest the involvement of the region between P*ori* and *ori*. Hillenbrand and Staudenbauer (7) reported that *in vitro* replication of ColE1-type plasmids was initiated in three regions of the plasmid molecules when RNase H was absent from the *in vitro* system. However, these sites were not near the normal origin (7). The reason for this discrepancy is not apparent, but it may be attributed to some specific conditions used in the *in vitro* system.

The results shown in Table 3 indicate that DNA polymerase I-independent replication is inhibited by addition of Rif. Since the replication does not require concomitant protein synthesis (Table 3), this indicates a direct involvement of DNA-dependent RNA polymerase in plasmid replication under *polA*$^-$ *rnh*$^-$ conditions. Furthermore, the plasmid replicates at a high copy number in *polA*$^-$ *rnh*$^-$ cells (Table 2) and DNA polymerase III is required for plasmid replication in *polA*$^-$ *rnh*$^-$ conditions (this work), as is the case for normal plasmid replication (35).

These similarities between the two replication systems suggest that the *polA*$^+$-independent pathway involves a modified version of the normal plasmid replication processes. I wish to suggest the following model as one of several schemes that are consistent with all the properties discussed above. In the absence of RNase H activity, the product of transcription initiated at P*ori* is allowed to form DNA·RNA hybrid in a much larger region downstream from *ori* than in the presence of the enzyme. The displaced single-stranded DNA is then stabilized, perhaps by RecA and/or Ssb protein (38, 39), resulting in an R loop, similar to the D-loop structure which is believed to be an early intermediate in homologous recombination (40). The *recA*$^+$-dependent plasmid concatemer formation observed in *rnh*$^-$ cells (16) may result from intermolecular recombination stimulated by the presence of such an R-loop structure. The R loop extends so far as to allow the entry of a replisome, thus bypassing the normal requirement for DNA polymerase I. This "R-loop" model predicts that switch-over from RNA to DNA occurs downstream from the normal origin, *ori*.

I thank Drs. B. Bachmann, N. Grindley, D. Smith, R. Crouch, and D. Natvig for bacterial strains and plasmids; and A. J. Clark and K. von Meyenburg for critically reading the manuscript. The excellent technical assistance of T. Torrey, J. Malone, and H. Snyder is greatly appreciated. This work was supported by Grant GM22092 and Minority Biomedical Science Program Grant PR08139 from the National Institutes of Health and by a grant-in-aid for research from the University of New Mexico.

1. Clewell, P. B. (1972) *J. Bacteriol.* **110**, 667–676.
2. Kingsbury, D. T. & Helinski, D. R. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1538–1544.
3. Staudenbauer, W. L. (1976) *Mol. Gen. Genet.* **149**, 151–158.
4. Tomizawa, J. (1978) in *DNA Synthesis: Present and Future*,

eds. Molineux, I. & Kohiyama, M. (Plenum, New York), pp. 797–826.
5. Arendes, J., Carl, P. & Sugino, A. (1982) *J. Biol. Chem.* **257,** 4719–4722.
6. Itoh, T. & Tomizawa, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2450–2454.
7. Hillenbrand, G. & Staudenbauer, W. L. (1982) *Nucleic Acids Res.* **10,** 833–853.
8. Kogoma, T. & von Meyenburg, K. (1983) *EMBO J.* **2,** 463–468.
9. Kogoma, T. (1978) *J. Mol. Biol.* **121,** 55–69.
10. Torrey, T. A. & Kogoma, T. (1982) *Mol. Gen. Genet.* **187,** 225–230.
11. Kogoma, T., Torrey, T. A., Subia, N. L. & Pickett, G. G. (1981) in *The Initiation of DNA Replication*, ICN–UCLA Symposia on Molecular and Cellular Biology, ed. Ray, D. (Academic, New York), Vol. 22, pp. 361–374.
12. de Massy, B., Fayet, O. & Kogoma, T. (1984) *J. Mol. Biol.* **178,** 227–236.
13. Ogawa, T., Pickett, G. G., Kogoma, T. & Kornberg, A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1040–1044.
14. Kanaya, S. & Crouch, R. J. (1983) *J. Biol. Chem.* **258,** 1276–1281.
15. Backman, K., Betlach, M., Boyer, H. W. & Yanofsky, S. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 69–76.
16. Kogoma, T. & Subia, N. L. (1983) in *Mechanism of DNA Replication and Recombination*, UCLA Symposia on Molecular and Cellular Biology, New Series, ed. Cozzarelli, N. R. (Liss, New York), Vol. 10, pp. 337–349.
17. Torrey, T. A., Atlung, T. & Kogoma, T. (1984) *Mol. Gen. Genet.* **196,** 350–355.
18. Kelley, W. S. (1980) *Genetics* **95,** 15–38.
19. Cooper, P. K. & Hanawalt, P. C. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1156–1160.
20. Tait, R. C., Harris, A. L. & Smith, D. W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 675–679.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
22. Barnes, W. M. & Beran, M. (1983) *Nucleic Acids Res.* **11,** 349–368.
23. Cohen, S. N. & Chang, A. C. Y. (1977) *J. Bacteriol.* **132,** 734–737.
24. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
26. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
27. Womble, D. D., Taylor, D. P. & Rownd, R. H. (1977) *J. Bacteriol.* **130,** 148–153.
28. Monk, M. & Kinross, J. (1972) *J. Bacteriol.* **109,** 971–978.
29. Timmis, K., Cabello, F. & Cohen, S. N. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2242–2246.
30. Hirota, Y., Gefter, M. & Mindich, L. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3238–3242.
31. Cabello, F., Timmis, K. & Cohen, S. N. (1976) *Nature (London)* **259,** 285–290.
32. Ogawa, T. & Okazaki, T. (1984) *Mol. Gen. Genet.* **193,** 231–237.
33. Itoh, T. & Tomizawa, J. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 409–417.
34. Clewell, D. B., Evenchik, B. & Cranston, J. W. (1972) *Nature (London) New Biol.* **237,** 29–31.
35. Collins, J., Williams, P. & Helinski, D. R. (1975) *Mol. Gen. Genet.* **136,** 273–289.
36. Masukata, H. & Tomizawa, J. (1984) *Cell* **36,** 513–522.
37. Naito, S., Kitani, T., Ogawa, T., Okazaki, T. & Uchida, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 550–554.
38. Shibata, T., DasGupta, C., Cunningham, R. P. & Radding, C. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1638–1642.
39. McEntee, K., Weinstock, G. M. & Lehman, I. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 857–861.
40. Dressler, D. & Potter, H. (1982) *Annu. Rev. Biochem.* **51,** 727–761.