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Illegitimate recombination occurs between the replication origin of the plasmid pC194 and a progressing replication fork

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Hybrids between plasmids pC194, pBR322 and the bacteriophage f1 undergo deletions in *Escherichia coli*. The deletions end most often between nucleotides 1445 and 1446 of pC194. That site probably corresponds to a nick in the replication origin of this plasmid. The localization of the other deletion end appears to be determined by the position of the f1 replication fork. Two models accounting for these data are discussed.
Key words: illegitimate recombination/generation of deletions/replication/plasmid pC194

Introduction

Considerations which were mostly of a theoretical nature led to the proposal that illegitimate recombination may be a consequence of errors of DNA replication, or of enzymes that break and join DNA molecules (Franklin, 1971). Experiments suggesting that both classes of errors may indeed occur were subsequently presented. For example, deletions in the *Escherichia coli lac* operon or in *Bacillus subtilis* plasmids, which take place between short repeated sequences, were attributed to replication errors (Albertini *et al.*, 1982; Ehrlich *et al.*, 1986). On the other hand, recombination observed *in vitro* between non-homologous sequences was suggested to be due to errors of breaking and joining activities of *E. coli* DNA gyrase (Ikeda *et al.*, 1981) or T4 DNA topoisomerase II (Ikeda, 1986). Similarly, eukaryotic DNA topoisomerases I were shown to break and join DNA segments *in vitro* (Been and Champoux, 1981; Halligan *et al.*, 1982) and were suggested to be involved in illegitimate recombination *in vivo* (Bullock *et al.*, 1984).

Recombination between sequences of little or no homology can also occur by breakage and reunion at a replication origin. Deletions formed by joining a nucleotide adjacent to the nick present in the replication origin of several filamentous single-stranded DNA phages (M13, f1, fd) to a distant nucleotide were described (Schaller, 1978; Horiuchi *et al.*, 1978; Michel and Ehrlich, 1986). Similarly, recombination at a preferred site (possibly a nick) in the transfer replication origin of the plasmid F was reported (Horowitz and Deonier, 1985). Recombination within the F vegetative replication origin has also been described (O'Connor and Malamy, 1984) and suggested to involve a nick (Michel and Ehrlich, 1986). These results indicate that either a structural feature present at different replication origins (i.e. a nick) or a functional feature of proteins involved in initiation of DNA replication (i.e. a nicking-closing activity) may be the cause of illegitimate recombination by breakage and reunion.

In the present work we studied deletions occurring at the replication origin of plasmid pC194, isolated from *Staphylococcus aureus* (Iordanescu, 1975). We show that one end of the deletions often coincided with a specific site in the replication region of pC194 which we believe corresponds to a nick. Furthermore, we show that the other end of the deletion was affected by the position of the f1 replication fork and we discuss models accounting for these observations.

Results

Replication origin of pC194 is a hot spot for deletion formation

Plasmids derived from pHV695. Plasmid pHV695 (Figure 1), constructed in *B. subtilis* where it is stably maintained, transformed *E. coli* competent cells harboring an F′ plasmid to ampicillin resistance with an efficiency of 10^5-10^6 transformants/μg of DNA. Of the transformants, 50% were Ap^R, 50% Ap^R Tc^R, as determined by replica-plating of 500 colonies.

Plasmid DNA was extracted from six Ap^R and 56 Ap^R Tc^R transformants and analysed by gel electrophoresis. Molecules of the size of pHV695 (13.6 kb) together with smaller molecules were found in >90% of the clones irrespective of the transformant phenotype. The remaining clones contained only smaller

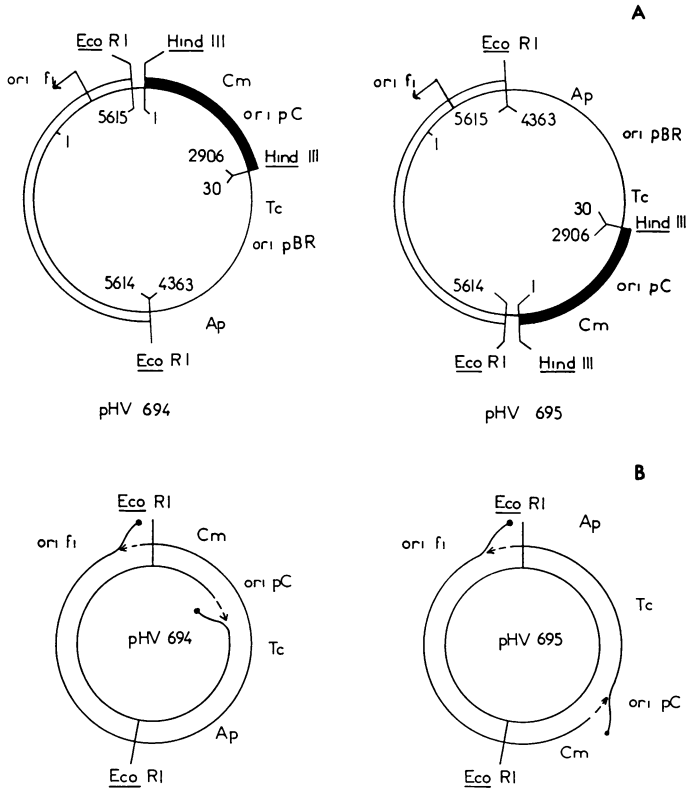

Fig. 1. (**A**) Schematic representation of pHV694 and pHV695. Thin, thick and double lines correspond to pBR322, pC194 and R229, respectively. The numbers refer to sequence coordinates (cf. Materials and methods). ori pBR, ori pC and ori f1 refer to the replication origins of pBR322, pC194 and R229, respectively. The restriction sites used for the construction are shown. The arrows indicate the direction of f1 replication. (**B**) Direction of replication initiated at pC194 and f1 origins in pHV694 and pHV695. The lines represent the DNA strands, dots and arrows represent 5′-phosphate and 3′-hydroxyl nucleotides, respectively.

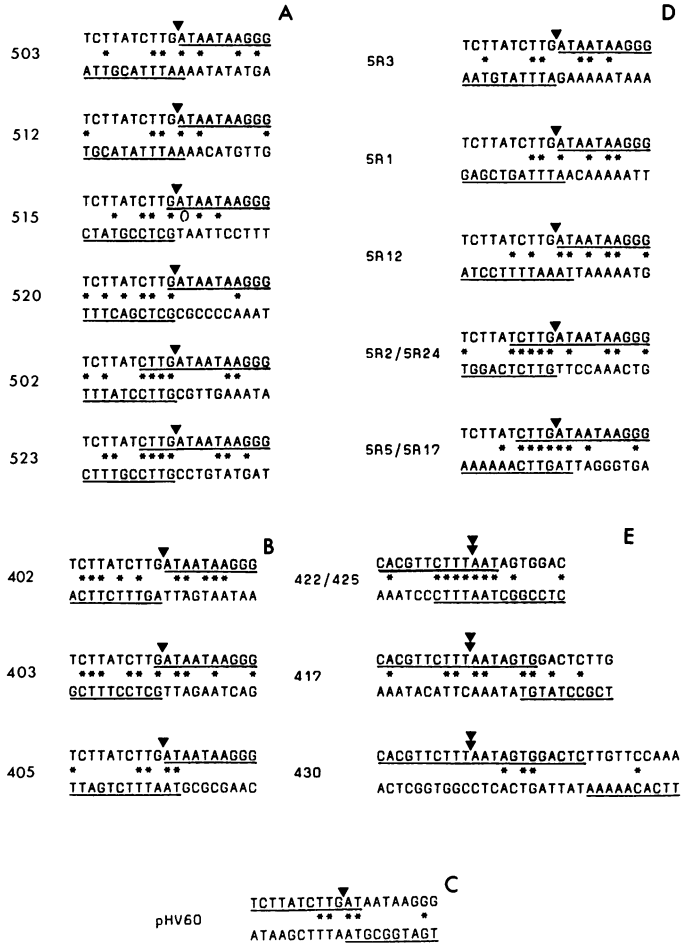

Fig. 2. DNA sequences involved in the recombination events. (**A**–**D**) The top sequence of each pair is that of pC194 origin (between nucleotides 1436 and 1455), the bottom strand that of the plasmid region where the recombination took place. The underlined sequence was determined by analyzing plasmids indicated by numbers, the triangle indicates the position of the nick. The site of recombination with the origin region was as follows. (**A**) pHV695 derivatives isolated in a Rep$^+$ strain: 503: 6234–6235 of f1; 512: 192–193 of f1; 515: 659 of f1; 520: 44 of f1; 502: 6265–6268 of f1; 523: 6382–6385 of f1. (**B**) pHV694 derivatives isolated in a Rep$^+$ strain: 402: 5287 of f1; 403: 5446 of f1; 405: 4999–5001 of f1. (**C**) pHV60: 37–38 of pBR322. (**D**) pHV695 derivatives isolated in a Rep$^-$ strain: 5R3: 4232 of pBR322; 5R1: 5895–5896 of f1; 5R12: 3236–3238 of pBR322; 5R2–5R24: 5791–5795 of f1; 5R5/5R17: 5697–5702 of f1. (**E**) pHV694 derivatives obtained in Rep$^+$ strain. The top line of each pair is that of the f1 origin, the arrow indicates the position of the nick (5880–5881). The site of recombination was as follows: 422–425: 5406–5412 of f1; 417: 4215–4216 of pBR322; 430: 5360 of f1.

plasmids. These observations indicate that pHV695 undergoes deletions with high frequency in *E. coli* and that its Cm^R and Tc^R genes do not confer upon that host a resistance to the antibiotic concentrations used.

Ap^R clones harbored molecules 8–10 kb long, which were composed of the phage sequences and the Ap^R gene of pBR322, but lacked the pBR322 replication origin as judged by: (i) restriction analysis; and (ii) their capacity to transfect and transform to Ap^R the F′ but not the isogenic F$^-$ *E. coli* cells. These molecules frequently underwent further deletions and were therefore not studied in more detail.

Small plasmids harbored in the Ap^R Tc^R clones were 5.8–6.8 kb in size. They were purified from pHV695 by transformation of *E. coli* F$^-$ competent cells and subjected to restriction analysis. In 26 of 29 analyzed plasmids one end of the deletion was localized between the *Mbo*II site at nucleotide 1356 and the *Hpa*II site at nucleotide 1465 of pC194. The remaining three plasmids, in which the deletion eliminated part of the pC194 A-protein, were not studied further. The 112-bp region in which most deletions ended has been previously shown to be necessary for pC194 replication (Dagert *et al.*, 1984). The deletion endpoints were sequenced in six of these plasmids. One site common to all the deletions, located between nucleotides 1445 and 1446 of pC194 was observed (Figure 2A). Very little sequence homology (1–4 bp) at the site of recombination was detected.

Plasmids derived from pHV694. Plasmid pHV694 (Figure 1) transformed *E. coli* F′ competent cells to Ap^R with an efficiency of 10^5–10^6 transformants/μg. By replica-plating 500 transformants were examined and found to be Ap^R, but their Cm or Tc phenotype could not be reliably ascertained.

Plasmid DNA was extracted from 51 transformants. In all cases several plasmid species were detected. One always matched the size of the parental plasmid (13.6 kb), the others ranged from 5 to 11 kb. This indicates that pHV694 is not stable in *E. coli*.

To purify individual plasmid species, 41 of the above DNA preparations were used to transform F$^-$ competent cells. Five to 50 Ap^R transformants were obtained in 32 cases, none in nine. These values are, on average, 200 times lower than those obtained with analogous preparations from pHV695-derived transformants, which suggests that most of the molecules smaller than pHV694 replicated as phages. The difficulty in determining the Tc and Cm phenotype of the host F′ cells may be due to that fact.

Transformants obtained with 14 DNA preparations were Ap^R Tc^R Cm^R and carried 7.5-kb plasmids. The structure of these is described in the last part of Results. Eighteen DNAs gave Ap^R Tc^R plasmids ~5.5 kb long. Twelve of these were analyzed by restriction. They resulted from a deletion ending between nucleotide 1356 and 1468 (an *Mbo*II and a *Hpa*II site) of pC194. Deletion endpoints were sequenced in three plasmids (Figure 2B). In one, no homology existed at the site of recombination, which could therefore be unambiguously assigned between nucleotides 1445 and 1446. In the other two, where 1- and 3-bp homology was found, the deletion could have taken place at the same site.

Inactivation of the A-protein of pC194. We wanted to determine whether inactivation of the gene A product, required for pC194 replication, would affect the site of deletion formation. Since the hybrids between R229 and a plasmid such as pHV33Δ81, in which the A-protein is inactivated, could not be constructed in *B. subtilis* (they would not replicate in that host, Dagert *et al.*, 1984), we used the following approach.

Phage R229 was linked *in vitro* to pHV33Δ81 and used to transform *E. coli* competent cells. The cells were cultivated for 2 h at 37°C in a liquid medium, centrifuged and discarded. The supernatant, which contained parental and hybrid phages was used to transduce *E. coli* F′ cells to Ap^R. Plasmids were extracted from seven transductants and analyzed by restriction. They were generated from a hybrid between R229 and pHV33Δ81 by deletions which in no case ended between nucleotides 1356 and 1468 of pC194. In contrast, 15 out of 22 plasmids isolated in an analogous way after ligating R229 to pHV33, which carries an intact gene A, were generated by deletions ending between nucleotides 1356 and 1468 of pC194.

The results presented in this section show that a majority of viable plasmids arising from pHV694 and pHV695 (55 and 90%,

respectively) were formed by deletions starting within the replication region of pC194, probably between nucleotides 1445 and 1446 and that inactivation of pC194 replication protein abolished deletion formation at that site. These results are analogous to those previously observed for M13, where deletions were occurring at the nick introduced in the phage replication origin by its gene II-encoded protein. They suggest that a nick is introduced in a region known to be necessary for plasmid replication (Dagert *et al.*, 1984) by the plasmid replication protein.

Plasmids derived from pHV14. As judged from the direction of replication of pC194 (te Riele *et al.*, 1986b), the sequences downstream from the putative nick in the pC194 replication origin (downstream and upstream from the nick refers to the region of the genome replicating early or late, respectively) are preserved in the genomes resulting from the deletions described above. These deletions must therefore end at the nucleotide located 3′ from that nick. The following evidence indicates that deletions may also preserve sequences upstream from the nick and presumably end at the nucleotide located 5′ from it.

Plasmid pHV14, composed of pBR322 and pC194, confers upon its host Ap^R and Cm^R but not the Tc^R phenotype, since the insertion inactivated the promoter of the Tc^R gene (Ehrlich, 1978). Tc^R plasmids appeared in strains carrying pHV14 with a frequency of 10^{-7}. Over 99% of these plasmids were generated by a deletion eliminating 1.4 kb of pC194 sequences and presumably fusing the Tc^R gene to a functional promotor. A representative Tc^R plasmid was named pHV32 (Primrose and Ehrlich, 1981). Deletion endpoints were sequenced in two independent isolates (I.Jones, personal communication). They were identical, and shared a 2-bp homology at the site of recombination (Figure 2C). The recombination event, which probably occurred between nucleotides 1445 and 1446 of pC194, preserved the sequences upstream from that site. Interestingly, a deletion event preserving sequences upstream from the same site and occurring in the presence but not in the absence of the A-protein, was recently observed in a hybrid between pC194 and a *Streptococcus pneumoniae* plasmid pLS2, propagated in *S. pneumoniae* (Ballester *et al.*, 1986).

Deletions at the phage replication fork

The results presented above suggest that one endpoint of deletions occurring in pHV695 corresponds to the nick in the pC194 replication region. Which factors determine the position of the other deletion endpoint? We observed with pHV695 that the deletions end in a 1.5-kb region downstream from the f1 replication origin (Figure 3A). The orientation of pC194 and f1 in pHV695 is such that the same strand of the hybrid molecule is nicked in the plasmid and the phage (Figure 1B). Formation of deletions in pHV695 could therefore depend on the joining of the nucleotide 5′-phosphate adjacent to the nick in pC194 to a nucleotide at the replication fork progressing from the nick in the f1 replication origin. To test that hypothesis we examined plasmids generated from pHV695 in a *rep*$^-$ host in which the helicase necessary for the phage replication is missing (Lane and Denhardt, 1974).

Plasmid pHV695 transformed the isogenic *rep*$^-$ and *rep*$^+$ cells to Ap^R with similar efficiencies. DNA was extracted from 24 *rep*$^-$ transformants. All contained plasmids smaller than pHV695, as judged by electrophoretic analysis. The parental plasmid could not be visualized by ethidium bromide staining, but could be detected by hybridization (not shown), whereas in 90% of the *rep*$^+$ transformants it could be detected by staining alone (see above). This indicates that the propagation of full size pHV695 in *E. coli* may depend on f1 replication functions.

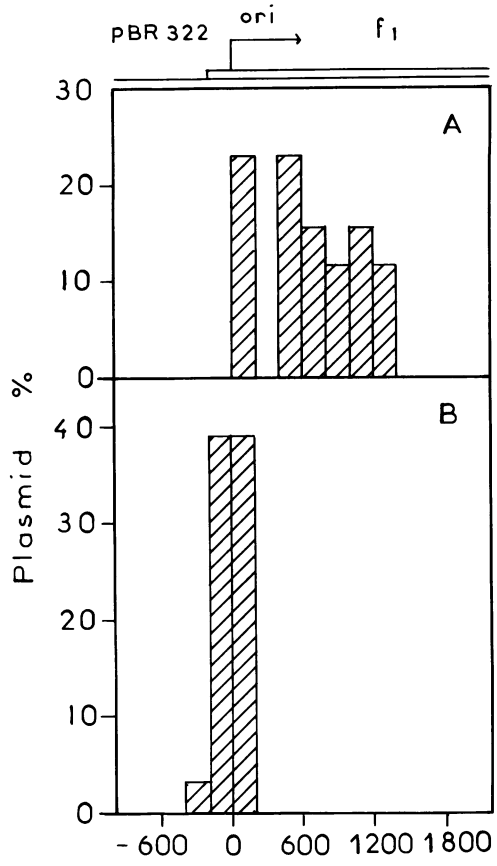

Fig. 3. Mapping of deletions ending close to the f1 replication region. Single and double lines at the top correspond to pBR322 and f1 sequences. The arrow indicates the direction of replication initiated at the f1 replication origin (ori). The zero of the abscissa coincides with the nick in the replication origin. The size of the intervals is 200 bp. **(A)** *rep*$^+$ host; **(B)** *rep*$^-$ host.

Of the 24 small plasmids isolated from the *rep*$^-$ cells, 23 (95%) resulted from a deletion ending between nucleotides 1356 and 1458 of pC194. Deletion endpoints were sequenced in seven plasmids (Figure 2D). Absence of homology at the site of recombination allowed us to unambiguously determine the deletion endpoint between nucleotides 1445 and 1446 in one plasmid. In six other plasmids where 1–6 bp homology was found between the recombining sequences the recombination could have occurred at the same site. This shows that the helicase lacking in the *rep*$^-$ host does not influence deletion formation at the pC194 nick.

The localization of the other deletion endpoint, determined by DNA sequencing in seven cases and restriction analysis in the remaining 16, is shown in Figure 3B. About 80% of the deletions ended in a 200-bp region, between 110 bp upstream and 90 bp downstream from the f1 replication origin. This is in sharp contrast to the localization observed in *rep*$^+$ cells (Figure 3A) where no deletion ended upstream from the nick, and ~75% of the deletions ended >400 bp downstream from it. The movement of the f1 replication fork appears to influence the localization of the deletion endpoint.

The importance of the orientation of the phage replication fork in deletion formation is illustrated by the analysis of pHV694. In this plasmid where complementary strands, rather than the same strand, are nicked in f1 and pC194 (Figure 1B) deletions starting at pC194 origin are 200 times less frequent than in pHV695 (see above)

Deletions at the f1 replication origin

Reduced frequency of deletion formation at the pC194 nick in pHV694 allowed us to detect deletions initiated at the replication origin of f1. Fourteen of 32 analyzed plasmids derived from pHV694 were ~7.5 kb in size, and conferred the Ap^R Cm^R Tc^R phenotype upon their host (see Results, first section). The size and resistance pattern indicated that deletions were not initiated at the nick in pC194. Eight plasmids examined in more detail were found to fall into four classes, each containing two plasmids indistinguishable by restriction analysis. The plasmids from the first class were formed by deletion ending within a 150-bp region upstream of the nick and were not analyzed further. Two plasmids from the second, one from the third and the fourth class were sequenced (Figure 2E). The two plasmids identical by restriction were formed by deletion occurring within a 7-bp sequence comprising the nick in the f1 replication origin. This event may be analogous to that previously observed to take place at the nucleotide located 5′ from the f1-carried nick (Schaller, 1978; Horiuchi *et al.*, 1978). In two other plasmids deletions took place 6 or 7 bp and 12 bp downstream from the nick. These results illustrate that deletions may be initiated at or in the vicinity of the f1 replication origin.

Discussion

Nick in the pC194 replication origin

The nick present in the replication origin of the *E. coli* single-stranded DNA phage M13 is the site at which deletions are frequently initiated (Michel and Ehrlich, 1986). We found in this work that a similar site exists in a region thought to contain the replication origin of pC194 (base pairs 1350–1650; Dagert *et al.*, 1984). Up to 95% of deletions isolated in a hybrid between the phage f1 and the plasmid pHV33 (composed of pBR322 and pC194, Primrose and Ehrlich, 1981) were initiated between nucleotides 1356 and 1465 of pC194. Sixteen deletions were analyzed by sequencing. Two could be localized unambiguously, since they presented no homology at the site of recombination. Both ended between nucleotides 1445 and 1446 of pC194. We found 1–6 bp homology at the site of recombination for 13 other deletions, which precluded unambiguous mapping. In all cases, however, one endpoint could have been situated between the same two nucleotides of pC194. Inactivation of the plasmid-encoded protein required for pC194 replication abolished the formation of deletions at that site. These results, strictly analogous with those obtained previously for deletions starting at the nick in the M13 replication origin, suggest that a nick is introduced in the replication origin of pC194 between nucleotides 1445 and 1446 by the plasmid replication protein.

Indirect support for the existence of a nick in the pC194 replication origin comes from the recent observations suggesting that this, and many other *B. subtilis/S. aureus* plasmids, replicate in a way analogous to that of single-stranded *E. coli* DNA phages. Briefly, circular single-stranded DNA appears to be a replication intermediate of the plasmids studied (te Riele *et al.*, 1986a,b; Gruss and Novick, 1986); a nick is known to be introduced *in vitro* in the replication origin by the replication protein of the best known of these, pT181 (Koepsel *et al.*, 1985).

The origin region of pC194 is rich in palindromes (Horinouchi and Weisblum, 1982). A part of that region is shown in Figure 4A. It can be folded in a hairpin with the stem and loop 5 and 2 bases long, respectively. The nick is introduced within the stem, 1 bp away from the loop. It is interesting that a sequence identical to the first 18 bp of the region represented in Figure 4A

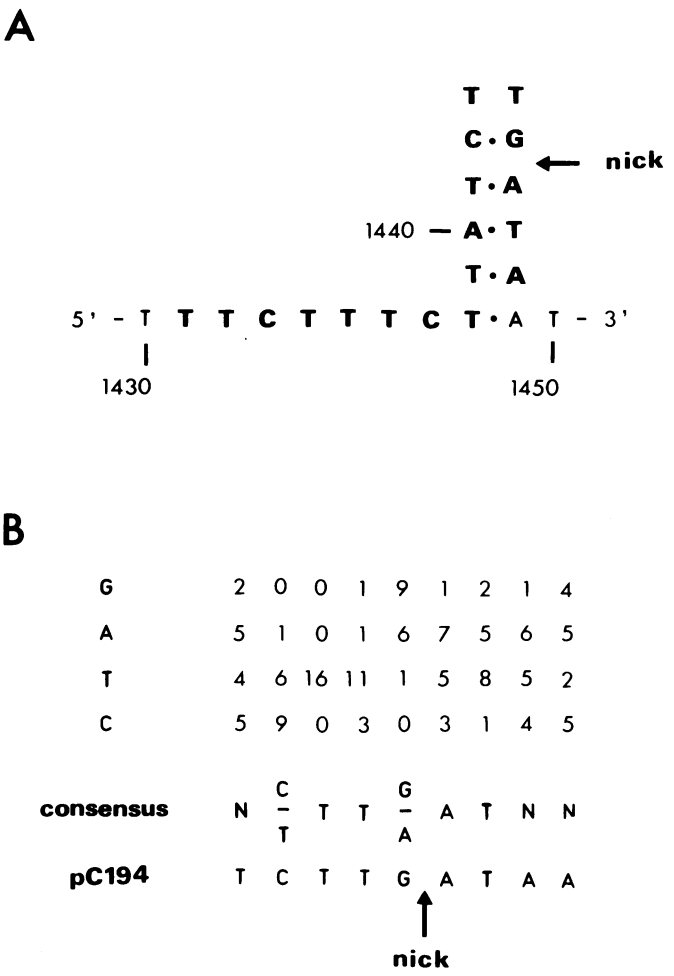

Fig. 4. (**A**) Replication origin of pC194. A nick is indicated by the arrow, the dots represent the potential hydrogen bonds. The 18-bp sequence present in the pUB110 replication origin is shown in heavy print. (**B**) Analysis of deletion endpoints. The values correspond to the number of times a given base was found at a given position within the plasmid sequence that had recombined with the pC194 replication origin (the 16 sequences displayed in Figure 2 were used). The consensus sequence was derived from the above values. The corresponding pC194 sequence is shown for comparison. The position of the nick is indicated by the arrow.

is found in the replication origin of pUB110 (Hahn and Dubnau, 1985). This suggests that pUB110 replicates in a way similar to that of pC194.

Mechanism of deletion formation

Plasmid pHV695 was constructed such that a nick is introduced in the same DNA strand at the f1 and the pC194 replication origins (Figure 1B). Deletions starting at the nick in pC194 ended in a 1.5-kb region downstream from the f1 origin when the plasmid host was permissive for phage replication (rep^+). They ended within 200 bp of the f1 origin when the host was not permissive (rep^-). This suggests that the position of the replication fork progressing from the phage replication origin affected the endpoint of deletions starting at the nick in pC194. Two models may account for that finding (Figure 5).

The A-protein errors. In single-stranded *E. coli* phages the synthesis of the viral strand is initiated and terminated by the action of a phage-encoded protein. Initiation consists of nicking a double-stranded DNA molecule while the termination consists of cleaving the nascent single strand from the replication intermediate

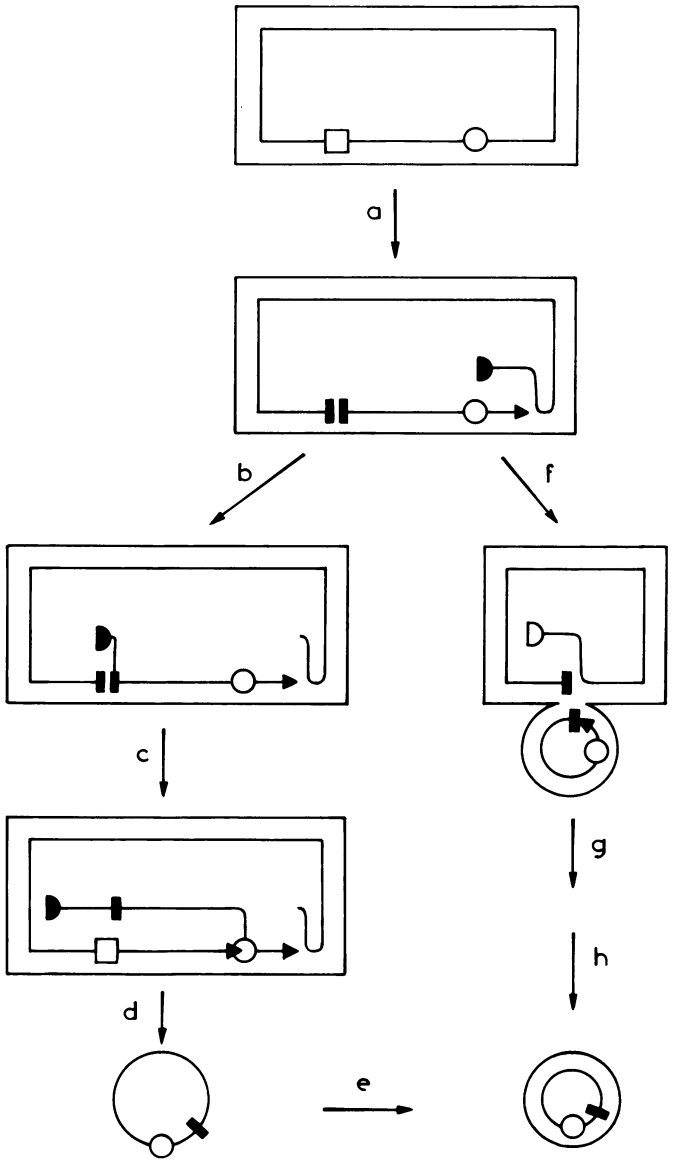

Fig. 5. The models of deletion formation at the nick. Open squares and circles symbolize intact pC194 and f1 replication origin, respectively. Filled symbols correspond to origin sequences upstream and downstream from the nick. Arrows indicate the 3′-hydroxyl nucleotide at the replication fork. (**a**) A nick is introduced in the pC194 replication origin and the replication is initiated at the f1 replication origin. (**b**) Single-stranded DNA displaced by the f1 replication fork is joined to the nucleotide located downstream from the nick in an erroneous termination reaction catalyzed by the pC194 A-protein. (**c**) The replication fork progressing from the pc194 replication origin has reached the f1 replication origin. (**d**) A single-stranded circular molecule is generated in a termination reaction catalyzed by the f1 gene II protein. (**e**) A double-stranded molecule is generated by the complementary strand DNA synthesis. (**f**) The nucleotide 5′-phosphate adjacent to the pC194 nick is joined to the 3′-hydroxyl nucleotide present at the replication fork by a putative DNA ligase. (**g,h**) A double-stranded circular DNA molecule is generated from the recombination intermediate either by DNA replication or by processing enzymes such as resolvase.

at the end of a round of replication and sealing it to form a covalently closed circular molecule (Eisenberg *et al.*, 1977; Horiuchi *et al.*, 1978; Meyer and Geider, 1982). Termination occurs at a specific signal, which partially overlaps the signal for initiation of DNA synthesis (Eisenberg *et al.*, 1977; Horiuchi *et al.*, 1978; Meyer and Geider, 1982). If the single-stranded replication intermediates of pC194 are generated in a way similar to that found for single-stranded DNA phages, the plasmid A-protein must have a cleaving–sealing activity and recognize a specific termination signal. It is then conceivable that deletions are formed in an erroneous termination reaction catalyzed by that protein as described in Figure 5a–d.

Erroneous termination could possibly occur at sequences resembling the correct termination signal, which should be related to those which flank the nicking site. By analysis of 16 deletions generated in this work we found similarities between the deletion endpoints and the pC194-carried hairpin within which the nick is introduced (Figure 4B). Two particularly striking features are that the T residue occurs invariably (16 out of 16 cases) or predominantly (11/16) at the positions corresponding to the loop of the hairpin, and that complementary bases always flank the two positions corresponding to the loop. Furthermore, A and T are present in the two positions 3′ from the nick in pC194 while A and T predominate in the two corresponding positions at the deletion endpoints. No obvious base pairing which could give rise to a hairpin similar to that seen in pC194 (Figure 4A) was, however, found for the last two nucleotides. The similarity between the deletion endpoints and the sequences flanking the nick supports the hypothesis that deletions occur in pC194 by erroneous termination of DNA synthesis.

It is interesting that deletions which start at the nucleotide located 5′ from the nick in pC194 (pHV60, Figure 2; pLS3, Ballester *et al.*, 1986), also end within the consensus sequence shown in Figure 4B. They could not result from erroneous termination, which occurs at the nucleotide located 3′ from the nick (cf. Figure 5). It is conceivable that they were generated by erroneous initiation of DNA replication within a sequence resembling that found in the pC194 origin, followed by displacement synthesis and the cleavage/joining reaction at the correct pC194 signal.

DNA ligase errors. An alternative model for deletion formation is based on a hypothesis that a nucleotide 5′-phosphate may be joined to a non-adjacent 3′-hydroxyl nucleotide, in a reaction resembling that observed with T4 ligase *in vitro* (Nilson and Magnusson, 1982). The nucleotide located 3′ from the pC194 nick, which probably carries a 5′-phosphate group, could be joined to the 3′-hydroxyl nucleotide present at the f1 replication fork (Figure 5f). The resulting recombination intermediate could give rise to the two reciprocal recombinants, or, if resolved by DNA replication, to one recombinant and one parental genome.

The known DNA ligases have no sequence specificity, and would not be expected to generate deletions ending in sequences resembling the pC194 replication origin. They could, however, be involved in the generation of deletions ending in sequences for which a common pattern was not found, such as those occurring at the replication origin of bacteriophages f1 (Figure 2E) or of M13 (Michel and Ehrlich, 1986).

Materials and methods

The bacterial strains, plasmids and phages used are shown in Table I. HVC749 was constructed by P1 transduction of D162 Rep3 mutation in HVC748. The plasmids pHV694 and pHV695 were constructed by joining *Eco*RI-cleaved R229 phage (Boeke, 1981) to pHV33 (Primrose and Ehrlich, 1981); they were isolated in *B. subtilis*.

Nucleotide coordinates used for pBR322 are from Sutcliffe (1978) as modified by Backman and Boyer (1983), for f1 from Beck and Zink (1981) and Hill and Petersen (1982) and for pC194 from Horinouchi and Weisblum (1982) modified and numbered from the *Hin*dIII site (Dagert *et al.*, 1984).

Plasmid DNA was extracted from *B. subtilis* and from *E. coli* by the clear lysate method (Niaudet and Ehrlich, 1979; Clewell and Helinski, 1969) and was purified by chromatography on hydroxyapatite columns (Colman *et al.*, 1978).

Table I. Bacteria, plasmids and phages

Name	Genetic markers	Origin or reference
Bacteria		
B. subtilis		
HVS49	*trp*C2 *his*A *aro*B2 *tyr*A	Ehrlich, 1978
E. coli		
D162	*thy lac leu* Rep3 su$^-$	Lane and Denhardt, 1974
HVC747	*thr*A1 *leu*6 *thi*1 *pro*A2 *his*-4 *arg*E3 *lac*Y1 *gal*K2 *ara*14 *xyl*-15 *mtl*-1 *tsx*-33 *rpl*-31 *sup*E44 *hsd*R	Michel and Ehrlich, 1986
HVC748	As HVC747 but F'*lac*I^q Z M15 pro$^+$	Michel and Ehrlich, 1986
HVC749	As HVC748 but Rep3	This work
Plasmids		
F'*lac*I^q Z	M15 *pro*$^+$	Boeke, 1981
pBR322		Bolivar *et al.*, 1977
pC194	Natural isolate	Iordanescu, 1975
pHV33	pBR322 linked to pC194	Primose and Ehrlich, 1981
pHV694	R229 linked to pHV33	This work
pHV695	As pHV694 but in opposite orientation	This work
Phages		
R208	f1-R199 linked to pBR322	Boeke *et al.*, 1979
R229	f1 derivative	Boeke, 1981

For analytical purposes, plasmid DNA was extracted from 1.5 ml overnight cultures (Birnboim and Doly, 1979). The terminal labeling method was used for DNA sequencing (Maxam and Gilbert, 1980). Transformation of *B. subtilis* and *E. coli* competent cells, and plasmid transduction were described previously (Niaudet and Ehrlich, 1979; Dagert and Ehrlich, 1979; Dagert *et al.*, 1984).

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