Repair of Double-Strand Breaks in Bacteriophage T4 by a Mechanism That Involves Extensive DNA Replication

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ABSTRACT

We investigated double-strand break (dsb) repair in bacteriophage T4 using a physical assay that involves a plasmid substrate with two inverted DNA segments. A dsb introduced into one repeat during a T4 infection induces efficient dsb repair using the second repeat as a template. This reaction is characterized by the following interesting features. First, the dsb induces a repair reaction that is directly coupled to extensive plasmid replication; the repaired/replicated product is in the form of long plasmid concatemers. Second, repair of the dsb site is frequently associated with exchange of flanking DNA. Third, the repair reaction is absolutely dependent on the products of genes *uvsX, uvsY, 32, 46,* and *59,* which are also required for phage genomic recombination-dependent DNA replication. Fourth, the coupled repair/replication reaction is only partly dependent on endonuclease VII (gp49), suggesting that either another Holliday-junction-cleaving activity or an alternate resolution pathway is active during T4 infections. Because this repair reaction is directly coupled to extensive replication, it cannot be explained by the SZOSTAK *et al.* model. We present and discuss a model for the coupled repair/replication reaction, called the extensive chromosome replication model for dsb repair.

DOUBLE-STRAND break (dsb) repair plays a key role in diverse biological events including meiosis, intron mobility, and VDJ recombination (reviewed in LAMBOWITZ and BELFORT 1993; JEGGO *et al.* 1995; SHINOHARA and OGAWA 1995). In addition, dsb repair has a major role in repairing DNA breaks created by a variety of compounds, including important anticancer and antibacterial agents (MCDANIEL *et al.* 1978; CALDECOTT *et al.* 1990; KREUZER 1994).

A popular family of models have been proposed to explain dsb repair, beginning with the model of RESNICK (1976), which was then modified by SZOSTAK *et al.* (1983) (Figure 1). The general features of these models include exonucleolytic degradation to expose 3′ ssDNA ends (step A), invasion of the 3′ ends into the homologous duplex DNA (step B), repair synthesis from the 3′ ends to replace the missing DNA (step C), and resolution of the two Holliday junctions (steps D and E). The net result is gene conversion at the site of the break (or gap), with or without exchange of flanking DNA. While this family of models has been successful in explaining many features of dsb repair, alternative models have also been proposed (KOBAYASHI 1992). Indeed, it is clear that no one model can explain all instances of dsb repair.

Most of the proteins likely to play a role in dsb repair in bacteriophage T4 have been purified and studied in the context of T4 DNA replication and recombination.

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T4 initiates DNA replication by two distinct modes (reviewed in MOSIG 1983; KREUZER and MORRICAL 1994). Early replication initiates from specific origin sequences on the chromosome by a mechanism that is independent of recombination proteins. As the infection progresses, the second mode, recombination-dependent replication (rdr), becomes predominant as the origins become repressed. T4 rdr requires phage-encoded recombination proteins and is believed to involve the conversion of recombination intermediates into replication forks. During rdr, T4 DNA is synthesized as long concatemers that are eventually packaged by a headful mechanism to generate circularly permuted, terminally redundant chromosomes (STREISINGER *et al.* 1964, 1967).

T4 rdr is closely related to recombinational repair (reviewed in KREUZER and DRAKE 1994) and thus provides a good starting point for understanding dsb repair in T4. Both rdr and recombinational repair require the products of genes *32, 46, 47, 59, uvsX,* and *uvsY,* along with T4-encoded replication proteins (reviewed in KREUZER and MORRICAL 1994; KREUZER and DRAKE 1994). A greater understanding of rdr has come through studies of an *in vitro* system that reconstitutes a portion of the reaction (FORMOSA and ALBERTS 1986; reviewed in KREUZER and MORRICAL 1994). In this system, a ssDNA primer triggers replication of a homologous duplex after a strand-invasion reaction. Strand invasion is promoted by UvsX (RecA homologue) along with its accessory protein UvsY and the ssDNA binding protein gp32. The helicase-primase complex (gp41/61) is loaded onto the synapsed recombination intermediate by gp59 (BARRY and ALBERTS 1994; MORRICAL *et al.*

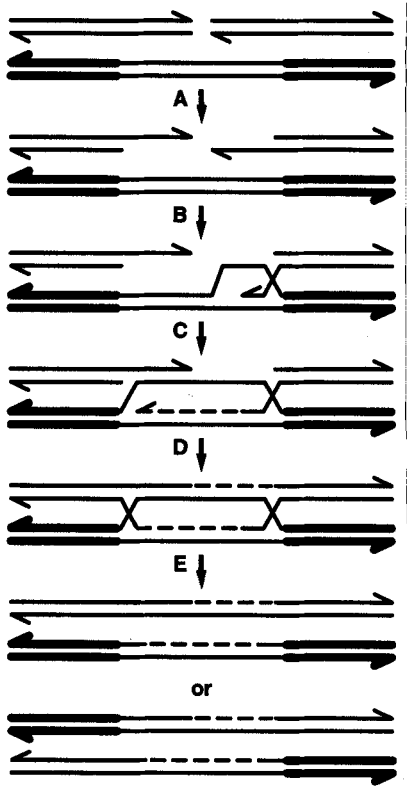

FIGURE 1.—The SZOSTAK *et al.* (1983) model for recombination-mediated dsb repair. (A) The DNA surrounding a dsb is degraded, predominantly by a 5′ to 3′ exonuclease, leaving 3′ single-stranded tails. (B) One 3′ tail invades intact homologous DNA, producing a D-loop. (C) Local replication enlarges the D-loop, exposing the complement of the second 3′ tail. (D) A second act of repair synthesis completes the top strand duplex. (E) The two Holliday structures are resolved by an endonuclease, producing either exchange or nonexchange for flanking DNA (only two of the four possible outcomes are shown). See SZOSTAK *et al.* (1983) for a more complete description.

1994), and DNA synthesis is then catalyzed by the T4 DNA polymerase holoenzyme complex (gp43/44/45/62) in a reaction that is stimulated by the phage-encoded type II DNA topoisomerase (gp39/52/60). Interestingly, this *in vitro* reaction shows no requirement for gp46 and gp47, although these proteins are required for rdr *in vivo*. One possible explanation is that gp46/47 is an exonuclease that is not necessary in the *in vitro* reaction because of the availability of the ssDNA primer.

T4 rdr has also been analyzed *in vivo* using a plasmid model system. Plasmids with homology to the T4 chromosome can be replicated during T4 infections (MATTSON *et al.* 1983), and this replication is an active process that requires the same phage-encoded proteins that are involved in phage genomic rdr (KREUZER *et al.* 1988b). The plasmid model system has recently been used to ask whether DNA ends can trigger rdr. Indeed, dsb's generated on the T4 chromosome stimulated the replication of a plasmid that is homologous to the broken region of the phage chromosome (KREUZER *et al.* 1995; see ASAI *et al.* 1994 for related work in the *Escherichia coli* system).

The opportunity to study dsb repair in T4 was increased by the discovery of mobile group I introns in the phage genome (reviewed in CLYMAN *et al.* 1994). Intron mobility depends on the generation of a dsb in an intron-minus allele by a site-specific endonuclease encoded within the intron of a coinfecting phage. In the dsb repair event that follows, the intron-containing gene serves as a repair template, resulting in acquisition of the intron by the gene that was previously intron free. The site-specific endonuclease encoded within the intron of the T4 *td* gene, called I-*Tev*I, has been purified and analyzed for DNA binding and cleavage site specificity (BELL-PEDERSEN *et al.* 1991; BRYK *et al.* 1993).

With the ability to introduce site-specific dsb's using I-*Tev*I and the extensive knowledge of recombination and replication proteins, T4 could provide an excellent system to study dsb repair. An additional advantage of the T4 system over nearly all others is the ease of determining the relationship between DNA repair and replication. During replication, T4 incorporates modified cytosine residues, making T4-replicated DNA refractory to most restriction endonucleases.

We decided to analyze dsb repair in the T4 system using a plasmid substrate that allows a simple physical assay for intramolecular repair events. Our choice for a plasmid substrate was based on the elegant work of I. KOBAYASHI and colleagues (YAMAMOTO *et al.* 1988). Using a plasmid containing inverted repeats, one of which sustains a dsb, several pathways of dsb repair in *E. coli* have been defined (KOBAYASHI 1992). In this report, we use a similar plasmid to analyze the repair of dsb's within inverted repeats during a T4 infection.

MATERIALS AND METHODS

Materials: Restriction enzymes, proteinase K, T4 DNA ligase and DNA polymerases were purchased from various commercial sources. The random primed labeling kit was obtained from Boehringer-Mannheim Biochemicals, α-^{32}P-dATP from Amersham, and nitrocellulose and Nytran membranes from Schleicher and Schuell. Oligonucleotides were synthesized by National Biosciences Inc. and by the Duke University Botany Department Oligonucleotide Synthesis Facility. L-broth contained Bacto-Tryptone (10 g/l), yeast extract (5 g/l) and sodium chloride (10 g/l). Plasmid-bearing cells were grown in L-broth with ampicillin (100 μg/ml). EHA plates for titering T4 contained Bacto-Tryptone (13 g/l), sodium chloride (8 g/l), sodium citrate (2 g/l), glucose (1.3 g/l) and agar (10 g/l).

Strains: *E. coli* strain KL16-99 (*recA1 relA spoT1 thi-1 deoB13*) has been described previously (LOW 1968). A spontaneous streptomycin-resistant mutant of KL16-99, called JG99S, was isolated by plating $\sim 5 \times 10^{10}$ cells on media containing streptomycin (200 μg/ml). The genotypes of T4 strain K10 and its derivatives are shown in Table 1. The *denA* and *denB* mutations in these phage strains prevent breakdown of host DNA (including plasmids); the amber mutations in genes *38* and *51* block phage assembly when not suppressed but have no effect on DNA metabolism (KUTTER *et al.* 1994).

Plasmids: Plasmid pIK43 (YAMAMOTO *et al.* 1988) was the generous gift of Dr. ICHIZO KOBAYASHI (University of Tokyo). Plasmid pJG43 was constructed by subcloning the 4738-bp *Sal*I/*Bam*HI fragment from pIK43 into *Sal*I/*Bam*HI-digested pUC19. Plasmid pJG43E was created by annealing two oligo-

TABLE 1

T4 strains

Strain	Genotype	Source
K10	*amB262* (gene *38*) *amS29* (gene *51*) *nd28* (*denA*) *rIIPT8* (a *denB-rII* deletion)	SELICK *et al.* (1988)
K10-*46*	K10, *amB14* (gene 46)	KREUZER *et al.* (1988a)
K10-*uvsX*	K10, *am11* (gene *uvsX*)	KREUZER *et al.* (1988a)
K10-*uvsY*	K10, *uvsYΔ1*	DERR and KREUZER (1988) KREUZER *et al.* (1988a)
K10-*32*	K10, *amA453* (gene *32*)	BENSON and KREUZER (1992)
K10-*59*	K10, *amHL628* (gene *59*)	KREUZER *et al.* (1988a)
K10-*49*	K10, *amE727* (gene *49*)	This work[a]

[a] T4 K10-*49* was generated from a genetic cross of the *amE727* single mutant (T4D background; BARTH *et al.* 1988) and T4 K10; see KREUZER *et al.* (1988a) for analogous constructions.

nucleotides comprising the I-*Tev*I cleavage site (BELL-PEDERSEN *et al.* 1991) flanked by *Xho*I ends (5′-TCGAGCTCAACGCTCAGTAGATGTTTTCTTGGGTCTACCGTTTAATATTGCGTCAC-3′ and 5′-TCGAGTGACGCAATATTAAACGGTAGACCCAAGAAAACATCTACTGAGCGTTGAGC-3′) into *Xho*I-cleaved pJG43. The I-*Tev*I cleavage site insert in pJG43E is oriented with the first oligonucleotide reading left to right (5′ to 3′) in the top segment (*i.e.*, as the top segment is drawn in Figure 2 below; determined by DNA sequencing). Plasmid pJGK43E was constructed by subcloning the 4794-bp *Sal*I/*Bam*HI fragment from pJG43E into the 10,057-bp *Sal*I/*Bam*HI fragment from pIK43.

Plasmids pJG1 (8645 bp) and pJG2 (8701 bp, see Figure 2) were constructed from pIK43 and pJGK43E, respectively, as follows. Each starting plasmid was digested with *Eco*RV (complete) and *Bgl*II (partial), and an 8645-bp (pJG1) or 8701-bp (pJG2) fragment was isolated. The 5′ overhangs at the *Bgl*II sites were then filled in using Klenow enzyme, and the resulting fragments were circularized by blunt-end ligation and transformed.

Plasmids pJG7 (8658 bp) and pJG8 (8714 bp) were constructed by digesting plasmids pJG1 and pJG2 with *Aat*II and ligating in a self-annealed oligonucleotide (5′-TTTTAATTAAAAACGT-3′) containing the octanucleotide *Pac*I cleavage site.

Plasmids pJG5 (8676 bp) and pJG6 (8732 bp) were constructed by digesting plasmids pJG7 and pJG8, respectively, with *Hin*dIII and then ligating in a self-annealed oligonucleotide (5′-AGCTATTTTAATTAATTAAAAT-3′) containing a *Pac*I and *Ase*I site.

dsb repair assay: Fresh overnight cultures of either KL16-99 or JG99S, containing the indicated plasmid substrates, were diluted into fresh L-broth and grown with vigorous shaking at 37° to a density of 4×10^8 cells/ml and then infected with the appropriate T4 strain at a multiplicity of three plaque-forming units/cell. After a 3-min incubation period at 37° without shaking to allow phage adsorption, infected cells were incubated for an additional 37 min at 37° with vigorous shaking.

Total nucleic acid was prepared as previously described (KREUZER *et al.* 1988a). Briefly, infected cells and any released phage particles from 1.5 ml of the infected culture were collected by centrifugation and resuspended in 300 μl lysis buffer [50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 100 mM NaCl, 0.2% SDS and proteinase K at 330 μg/ml]. The resuspended samples were incubated at 65° for 1 hr, extracted sequentially with phenol, phenol/chloroform and chloroform, and dialyzed against TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA] overnight at 4°.

Total nucleic acid (12–18 μl) was digested with the indicated restriction enzyme(s), treated with proteinase K (100 μg/ml) and SDS (0.1%) at 65° for 1 hr and subjected to electrophoresis at 70 V for 16 hr in a 1% agarose gel (13 cm × 25 cm) cast in 1× TBE (89 mM Tris, 89 mM boric acid and 2 mM EDTA). Southern blotting was then performed using either nitrocellulose or Nytran membranes using procedures recommended by the supplier (Schleicher and Schuell). The probe (usually pJG2 DNA, unless otherwise indicated) was prepared by incorporation of α-^{32}P-dATP using the random-primed labeling kit (Boehringer-Mannheim Biochemicals).

Field inversion gel electrophoresis: The structure of plasmid DNA that had been packaged into phage particles was analyzed as follows. KL16-99 cells harboring plasmid pJG8 were infected with T4 K10 as described above. After addition of chloroform to complete cell lysis, 1.5 ml of the lysate containing the packaged phage and cellular debris was centrifuged at 16,600 × *g* in a microcentrifuge for 1 min to remove cell debris. The cleared supernatant was centrifuged at 16,600 × *g* in a microcentrifuge for 1 hr (4°) to pellet the phage particles, which were resuspended in 300 μl of lysis buffer. DNA was prepared by proteinase K treatment, extraction and dialysis as described above.

Field-inversion gel electrophoresis was performed using a PPI-200 programmable electrophoresis controller (C.M.J. Research Inc.). *Pac*I-digested DNA products were resolved in a 0.8% agarose gel (13 × 25 cm) cast in 0.5× TBE containing ethidium bromide (0.5 μg/ml) at room temperature with constant recirculation. Electrophoresis was performed at 150 V for 24 hr with ~180 repeating 8 min-7 sec cycles. Each cycle consisted of alternating forward and reverse time ramps that increased, in an exponential fashion, from 0.15 to 12.03 sec (forward) and 0.05 to 4.01 sec (reverse) (program #4 from the manufacturer's operating manual). The DNA was analyzed by Southern blotting with a radiolabeled pJG2 probe as described above.

PCR amplification of plasmid DNA: Probes for the 283-bp *Nae*I fragment and the 248-bp *Nar*I fragment were prepared by amplification using a Perkin-Elmer DNA Thermocycler. A 237-bp subfragment of the 283-bp *Nae*I fragment was amplified using the oligonucleotides 5′-GGGTGTGGCGGACCGCTATCAGGAC-3′ and 5′-GGCGGCGGTGGAATCGAAATCTCGT-3′. A 147-bp subfragment of the 248-bp *Nar*I fragment was amplified using the oligonucleotides 5′-GCGCTGACAGCCGGAACA-3′ and 5′-GAGACAGGATGAGGATCGTT-3′. The PCR products were gel purified and then radiolabeled by incorporating α-^{32}P-dATP using the random primed labeling kit (Boerhinger-Mannheim Biochemicals).

RESULTS

dsb repair assay: A plasmid-based assay was developed to analyze dsb repair during phage T4 infections. Plasmid pJG2 contains two nearly identical segments (~2000 bp each), originally from the *kan* gene of Tn*5*, in an inverted orientation with respect to one another (Figure 2). The homologous segments differ at two locations. First, the middle of the bottom segment contains a 283-bp *Nae*I fragment (a) that is replaced in the top segment by a 56-bp fragment containing the I-*Tev*I cleavage site (A). Second, the top segment contains a 248-bp *Nar*I fragment (B) that is missing in the bottom segment (b). Upon T4 infection, a dsb is created at the I-*Tev*I cleavage site (A) by the phage-encoded I-*Tev*I endonuclease. Repair of the dsb is monitored by Southern hybridization, using the characteristic changes in *Ase*I restriction fragments. We will use the terms "conversion" and "coconversion" to describe the events and products that occur when the dsb in the top segment is repaired using information from the bottom segment of the plasmid. We acknowledge that we have not met the strict definition of "gene conversion" by counting all products of the repair reaction. Nonetheless, a mechanism that qualifies as a true gene conversion process is extremely likely, given that marker A sustains a dsb and is presumably lost during the repair process.

We also determined whether the repaired product has been replicated by T4 by performing *Ase*I-*Hae*III double digests. While *Ase*I cleaves DNA regardless of the presence of cytosine modifications, *Hae*III cannot digest the glucosylated hydroxymethylcytosine-containing DNA resulting from T4-directed replication (KREUZER *et al.* 1988a; KUTTER *et al.* 1994). Because the plasmid contains numerous *Hae*III sites, *Ase*I fragments that have not been replicated by T4 are digested into much smaller fragments, whereas T4-replicated *Ase*I fragments are refractory to *Hae*III. Also, T4-replicated DNA has a slower mobility on agarose gels due to the glucosyl residues of the modified cytosines (KREUZER *et al.* 1988a).

Based on the SZOSTAK *et al.* (1983) model for dsb repair, the expected repair products and the predicted *Ase*I fragment sizes are presented in Figure 2. In product 1, conversion of the dsb site (A) in the recipient DNA (top) results in the acquisition of a from the donor DNA (bottom) and thus generates a unique 4148-bp *Ase*I fragment from the 3921-bp parental fragment. Product 2 is generated by coconversion of A to a and B to b, yielding a unique *Ase*I fragment of 3900 bp. In this plasmid construct, the expected *Ase*I fragments are identical whether or not there has been an exchange of the flanking DNA segments. Therefore, only the nonexchange products are shown in Figure 2.

dsb repair and associated replication: We began these experiments with T4 strain K10, which lacks the DenA and DenB endonucleases that normally degrade host DNA including plasmids (KUTTER *et al.* 1994). *E. coli* KL16-99 cells harboring either pJG2 (Figure 2) or pJG1 (a control plasmid lacking the I-*Tev*I cleavage site) were infected, and DNA samples were prepared at various times after infection. In the uninfected controls, *Ase*I

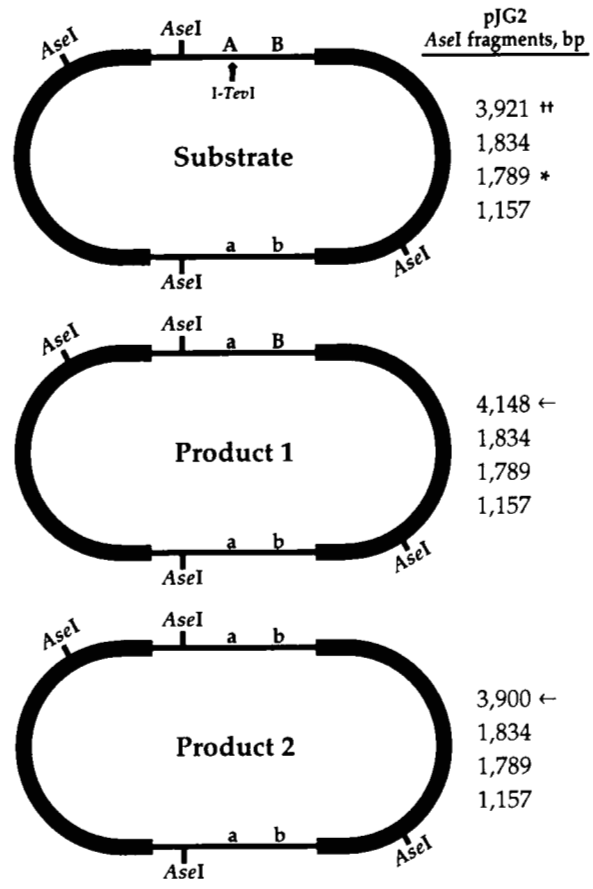

FIGURE 2.—The starting plasmid substrate and predicted repair products. (A) The two nearly identical homologous segments of pJG2 (coordinates 3-2121 and 4337-6436; coordinates are clockwise and relative to the *Eco*RI site, which is located at the border of the Q R S segment and the A B segment; also see YAMAMOTO *et al.* 1988) are shown as thin parallel lines. The upper segment has a 56-bp DNA fragment containing the I-*Tev*I cleavage site (A), while the bottom segment has an unrelated 283-bp fragment (a) at the same location. The upper segment has a 248-bp fragment (B) not present in the bottom segment (site of missing fragment is labeled b). Within each segment, the two heterologies are separated by 506 bp of homology and are flanked to the left by 1079 bp and to the right by 231 bp of homologous DNA. The cleavage sites for restriction enzyme *Ase*I are shown, along with the predicted sizes of the cleavage products. The 3921-bp (++) and 1789-bp (*) *Ase*I DNA fragments contain the recipient (*i.e.*, cleaved by I-*Tev*I) and donor DNA, respectively. Product 1 results from conversion at A (loss of the I-*Tev*I cleavage site fragment A and inheritance of the 283-bp fragment a), producing a new 4148-bp *Ase*I fragment (arrow) from the 3921-bp recipient *Ase*I fragment of the substrate. Product 2 is generated when the right side of the dsb is degraded beyond the B/b heterology. In this case, conversion of A to a is accompanied by coconversion of B to b, resulting in a novel 3900-bp *Ase*I fragment (arrow). The two thick segments of plasmid vector sequence are not homologous to each other.

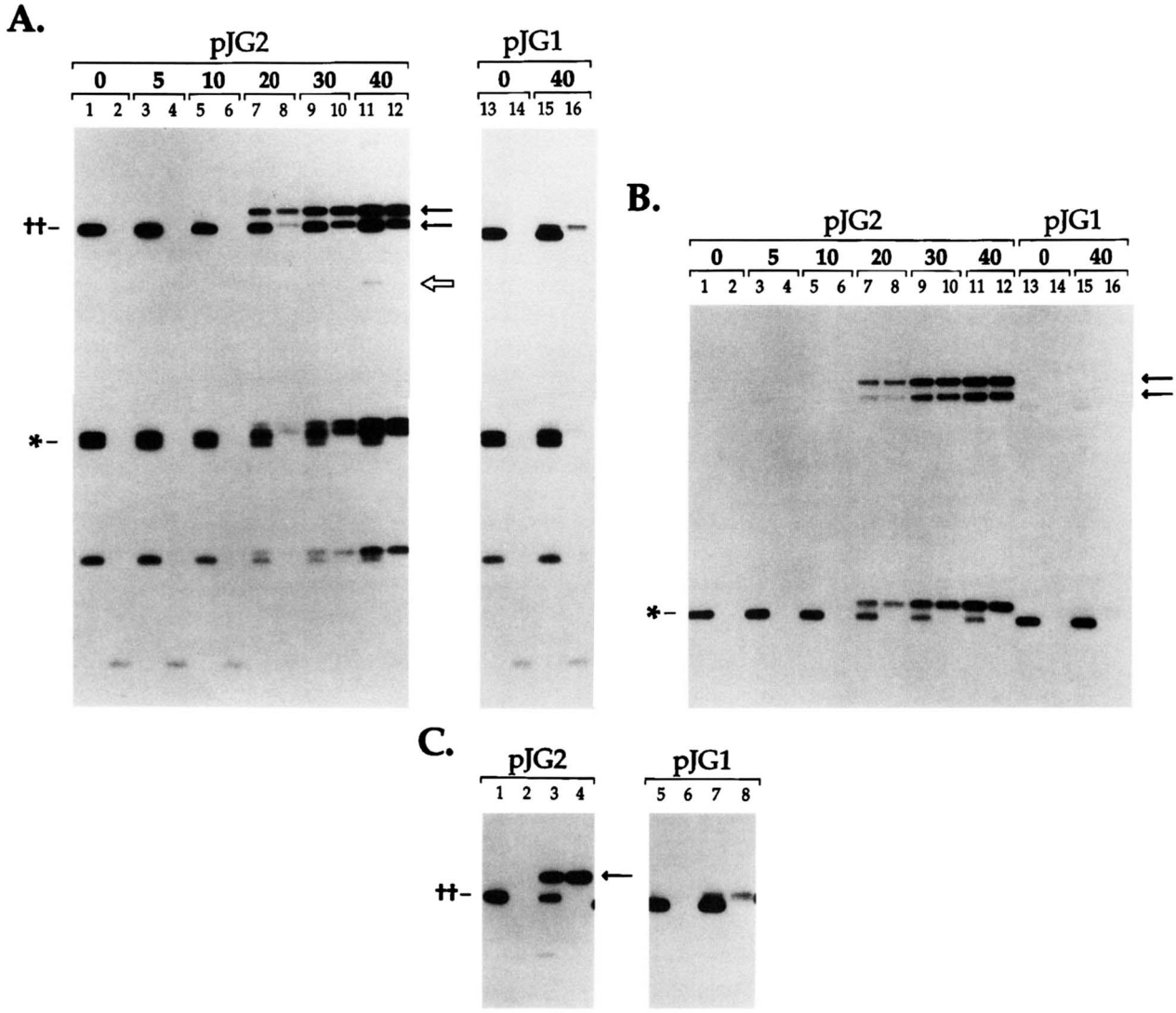

FIGURE 3.—Time course of dsb repair in wild-type infections. The time (min post infection) when each DNA sample was harvested is indicated above the lane numbers. The zero time points were from uninfected cells. The samples were digested with *Ase*I in the odd-numbered lanes, and with *Ase*I and *Hae*III in the even-numbered lanes. In A, the time-course digests were probed with radiolabelled pJG2 DNA. The recipient and donor fragments (see text) are indicated by †† and *, respectively. The conversion products are indicated by solid arrows, and the I-*Tev*I-cleaved substrate fragment by an open arrow. Internal size markers are provided by *Ase*I-cleaved pJG2 DNA from uninfected cells (lane 1), which yields fragments of 3921, 1834, 1789, and 1157 bp. The migration of all fragments was also compared to *Xba*I fragments of T4 cytosine-containing DNA for accurate sizing. In even-numbered lanes, the largest *Hae*III restriction fragment (815 bp) of the unreplicated (unmodified) plasmid is visible near the bottom of the gel. In B, the same time-course digests were probed with a 237-bp segment of DNA from within segment a (see Figure 2). In C, the digests were probed with a 147-bp segment of DNA from within segment B (see Figure 2). Note that replicated plasmid restriction fragments migrate slower than their unreplicated counterparts due to the cytosine modifications introduced during replication.

digestion of the DNA from either plasmid-bearing host produced the four expected *Ase*I fragments (Figure 3A, lanes 1 and 13). The 1789-bp fragment (*) contains the donor DNA for dsb repair and the 3921-bp fragment of pJG2 (++) is the *Ase*I fragment containing the recipient DNA with the I-*Tev*I cleavage site. As expected, *Hae*III digested the fragments from uninfected cells into small fragments (Figure 3A, lanes 2 and 14).

As the infection of pJG2-containing cells progressed, several *Hae*III-resistant *Ase*I fragments became prominent. The products most relevant to the dsb repair reaction include a fragment that is consistent in size with conversion product 1 (4148 bp) and an ~3900-bp fragment that represents either replicated parental DNA or conversion product 2 (Figure 3A, lanes 7–12, arrows). Note that *Hae*III-resistant bands migrate slower than their unreplicated counterparts, due to the glucosylated cytosine residues from T4-directed replication.

One of the most interesting observations from this experiment is that the dsb at A stimulated replication of plasmid pJG2 well above the level observed in the pJG1 control (which lacks the I-*Tev*I cleavage site; compare Figure 3A, lanes 12 and 16). Direct radioisotope counting of the blot revealed that the dsb stimulated

plasmid replication approximately sevenfold (data not shown). Replication of the entire length of the repaired plasmid is not predicted by the SZOSTAK *et al.* (1983) model (see DISCUSSION).

The I-*Tev*I-cleaved plasmid DNA was also detected in this experiment. A new 3161-bp *Ase*I fragment, the larger product of I-*Tev*I cleavage at A, was faintly visible at the 30- and 40-min time points (Figure 3A, lanes 9 and 11; open arrow). The smaller 760-bp product hybridizes to a much lower level because of its small size and therefore is not visible in this exposure. No T4-replicated (*Hae*III-resistant) I-*Tev*I cleavage product was observed at any time point (Figure 3A, even lanes).

To further characterize the repair products shown in Figure 3A, the same samples were probed with a DNA fragment from within the 283-bp *Nae*I fragment (a in Figure 2). In the uninfected control DNA, the probe recognized the 1789-bp *Ase*I fragment (donor, *) as expected and cross hybridized very weakly to the parental 3921-bp *Ase*I fragment (Figure 3B, lanes 1 and 13). If conversion were responsible for the 4148-bp and 3900-bp *Hae*III-resistant *Ase*I fragments described above, then both should be recognized by the a probe. Indeed, the a probe hybridized to both fragments (Figure 3B, lanes 7–12), indicating that these are *bona fide* conversion product 1 and 2. Conversion product 1 and 2 were not detected in the pJG1 control, demonstrating that conversion is strongly dependent on the introduced dsb.

The identities of the conversion products were also confirmed by probing the 0-min (uninfected) and 40-min DNA samples with PCR-generated DNA from the 248-bp *Nar*I fragment (B). As expected, the probe recognized the 3921-bp (*Hae*III-sensitive) *Ase*I fragment of the substrate pJG2 in the uninfected control (Figure 3C, lane 1). In samples containing the products generated from pJG2 after T4 infection, the B probe recognized only the 4148-bp *Ase*I fragment (Figure 3C, lane 4), consistent with the assignment of this band as conversion product 1. The B probe did not hybridize to the 3900-bp *Hae*III-resistant *Ase*I fragment that had been detected with the other probes, strongly arguing that this band is replicated conversion product 2 rather than replicated parental DNA. Finally, the small amount of replicated control plasmid (pJG1) DNA hybridized to the B probe (Figure 3C, lane 8), as expected for DNA that has not undergone conversion.

Together, these results demonstrate that the introduced dsb within segment A results in a dramatic increase in plasmid DNA replication. Essentially all of the replicated plasmid DNA is in the form of two distinct conversion products, one with conversion only at the A site (product 1) and the other with coconversion of the neighboring B site (product 2).

Exchange of flanking DNA segments: Certain dsb repair models (*e.g.*, SZOSTAK *et al.* 1983) predict that repair is accompanied by exchange of flanking DNA segments half of the time (see Figure 1). To determine if exchanges occur in the T4 repair/replication reaction, two *Pac*I sites were introduced into pJG1 and pJG2 at positions flanking the top homologous DNA cassette, generating plasmids pJG5 and pJG6 (Figure 4A). The predicted *Pac*I digestion products for product 1 DNA, with and without associated exchange of flanking DNA, are shown in Figure 4A (see figure legend for the predicted product 2 *Pac*I fragments). DNA was prepared from JG99S cells containing pJG5 or pJG6, either without infection or 40 min after infection by T4 strain K10. DNA from the pJG6-containing cells generated *Pac*I fragments consistent in size with both exchange and nonexchange products (Figure 4B, lanes 3 and 4). Direct radioisotope counting of the filter, with appropriate correction for size, revealed that exchange products comprised ~50% of both conversion product 1 and 2. Exchange products were not detected without T4 infection or without the I-*Tev*I dsb site (Figure 4B, lanes 1 and 2 and 5–8). These results demonstrate that the coupled repair-replication reaction involves conversion of the dsb with associated exchange of flanking DNA ~50% of the time.

Repaired plasmid DNA is concatemeric: Plasmids that are replicated by the recombination-dependent mode of T4 DNA synthesis were previously shown to be in the form of long concatemers that could be packaged by T4 (KREUZER *et al.* 1988b). Since the dsb repair reaction is accompanied by plasmid DNA replication, we determined whether the replicated plasmid DNA was also in the form of long concatemers.

DNA was isolated from phage particles produced in T4 K10 infections of JG99S cells containing plasmid pJG8. This plasmid contains a single *Pac*I site in the nonrepeated Q R S segment but is otherwise identical to pJG2 (Figure 5B, top). The isolated DNA was digested with *Pac*I, *Pac*I plus *Hae*III, *Hae*III, or no enzyme and was analyzed by field-inversion gel electrophoresis followed by Southern blotting with a plasmid probe. As a control, *Pac*I-digested pJG8 DNA from uninfected cells produced the expected single product of 8714 bp (Figure 5A, lane 1).

Both the uncut packaged plasmid DNA (Figure 5A, lane 5) and the *Hae*III-treated DNA (Figure 5A, lane 4) migrated as a single band above the 145-kb lambda DNA marker. In the course of its infection, T4 packages 170-kb linear DNA fragments by the headful mechanism. Further characterization revealed that the uncut packaged plasmid DNA migrates as a single band between 145- and 194-kb markers and comigrates with packaged T4 genomic DNA (data not shown). Therefore, the size of the observed plasmid DNA band is consistent with plasmid replication generating very long (>170 kb) concatemeric products, which are then cleaved into 170-kb fragments during DNA packaging.

Because the plasmid used in this experiment has a single *Pac*I site, the arrangement of adjacent monomers in the concatemeric plasmid DNA can be deduced by analyzing *Pac*I digests. With packaged DNA that had

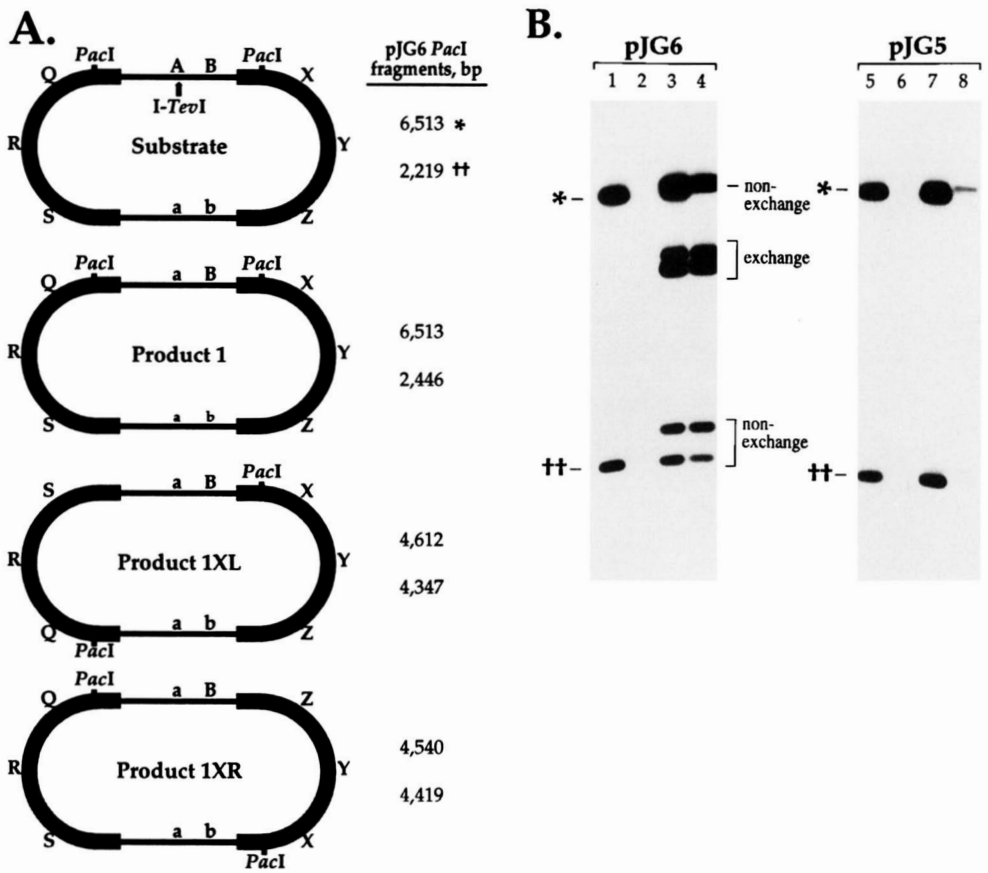

FIGURE 4.—Exchange of flanking DNA in the repaired/replicated product. (A) Illustrations of the pJG6 plasmid substrate and conversion product 1 DNA are presented, along with the predicted *Pad* digestion products. Nonhomologous DNA on the left and right sides of the plasmid are indicated by Q R S (coordinates 6398-8732) and X Y Z (coordinates 2065-4299), respectively. The three products shown have no exchange (product 1), an exchange on the left flank (*i.e.*, flipping segment Q R S; product 1XL), or an exchange on the right flank (*i.e.*, flipping segment X Y Z; product 1XR). The predicted sizes for the corresponding product 2 molecules are as follows: 6513 and 2198 bp (nonexchange); 4364 and 4347 bp (2XL); and 4419 and 4292 bp (2XR). Plasmid pJG5 is identical to pJG6 except that pJG5 does not contain the I-*Tev*I site insert. (B) Total nucleic acid was isolated from uninfected cells (lanes 1, 2, 5 and 6) or from cells 40 min after infection with T4 K10 (lanes 3, 4, 7 and 8). The samples were digested with *Pad* in the odd-numbered lanes and with *Pad* and *Hae*III in the even-numbered lanes. The filter was probed with radiolabeled pJG6 DNA. In both parts of this figure, the recipient and donor fragments are indicated by †† and an *, respectively. Internal size markers are provided by *Pad*-cleaved pJG6 DNA from uninfected cells (lane 1), which yields fragments of 6513 and 2219 bp. The migration of all fragments was also compared to λ *Hin*dIII markers for accurate sizing (data not shown).

been digested with *Pad*, five major bands were revealed by the plasmid probe (Figure 5A, lane 2 and data not shown). One band comigrated with the 8714-bp starting plasmid and disappeared when *Hae*III was present (Figure 5A, lane 3), indicating that it consists of unreplicated plasmid DNA. We suspect that this unreplicated plasmid DNA is a contaminant in the phage DNA preparation, but we cannot rule out the possibility that it was packaged within phage particles. The four other bands in the *Pad* digest (indicated by arrows) were each resistant to *Hae*III (Figure 5A, compare lanes 2 and 3) and therefore had been replicated by T4. The cytosine modifications characteristic of T4-replicated DNA result in a substantial decrease in migration in this kind of field-inversion gel (data not shown). We also analyzed the same digests on a standard (noninverting) gel, where the mobility change is less pronounced. The migration of the four bands was consistent with the sizes expected from the following interpretations; the bands are labeled according to their size relative to the unit length (UL) of the monomeric products (Figure 5; >UL, =UL, <UL). We assign the largest band as a doublet of 11,081-bp and 10,833-bp fragments resulting from adjacent exchange/nonexchange products in the same concatemer (Figure 5B, >UL; exchange flips the orientation of the Q R S segment with the *Pad* site). The two different sizes (11,081 and 10,833 bp) of the >UL product are expected from the inheritance of either marker B or b during the repair event (equivalent to products 1 or 2 in the above experiments). Similarly, the smallest pair of fragments (predicted sizes 6749 and 6501 bp) would result from an adjacent nonexchange/exchange arrangement with either B or b (Figure 5B, <UL). We deduce that the second largest band is a doublet of 8915- and 8667-bp fragments that result from adjacent monomers that are both exchange or that are

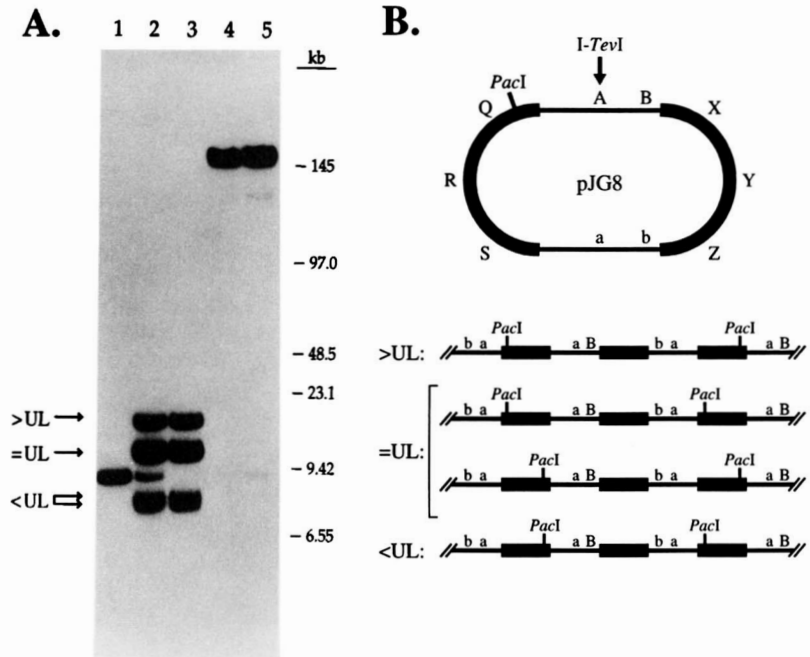

FIGURE 5.—Field-inversion gel electrophoresis of repaired pJG8 DNA. (A) Field-inversion gel electrophoresis was performed on packaged DNA isolated from pJG8-containing cells infected with T4 K10 (see MATERIALS AND METHODS). Lane 2 contains a *Pac*I digest of the packaged DNA, lane 3 contains a *Pac*I/*Hae*III double digest, lane 4 contains a *Hae*III digest and lane 5 contains uncut packaged DNA. Lane 1 contains the control of *Pac*I-digested plasmid DNA isolated from uninfected cells. The dashes on the right indicate the positions of lambda marker DNA fragments (concatemers and a *Hin*dIII digest). The transferred gel was probed with radiolabeled pJG2 DNA. (B) The top diagram shows the pJG8 plasmid substrate. The deduced arrangements of adjacent monomeric units within the plasmid concatemers are illustrated below the plasmid substrate and are labeled according to the *Pac*I fragment size (>, =, or <) relative to the unit length (UL) of the monomeric products. An exchange event flips the Q R S segment (relative to the adjacent b a and a B segments), changing the position of the *Pac*I site. The exchange state of the Q R S segment, from left to right, in the four diagrams are as follows: >UL, exchange/nonexchange; =UL, exchange/exchange (top) and nonexchange/nonexchange (bottom); <UL, nonexchange/exchange.

both nonexchange, again with inheritance of either B or b (Figure 5B, =UL).

The fractional amounts of the various products determined by direct radioisotope counting of the nylon filter from Figure 5A were found to be about 0.21:0.54:0.25 (>UL: = UL:<UL, respectively, without regard for marker B or b). This relationship reveals that neighboring monomers within the concatemer have a roughly equal probability of being in the same or opposite exchange configuration (see DISCUSSION). This finding is also consistent with the observation from the experiment in Figure 4 that exchange of flanking DNA has occurred in ~50% of the products.

Together, these results demonstrate that the dsb-induced plasmid replication generates long plasmid concatemers in which every constituent monomeric unit is replicated in its entirety. These results thereby contradict the model of SZOSTAK *et al.* (1983), in which replication is constrained to homologous DNA surrounding the break, and argue that rdr and recombination-mediated dsb repair in bacteriophage T4 function by an identical mechanism.

Protein requirements: T4 rdr and recombinational repair both require the products of genes *32, 46, 47, 59, uvsX* and *uvsY* (see Introduction). To test the requirements for these genes in the dsb repair/replication system, T4 K10 derivatives with single amber or deletion mutations were used to infect cells harboring plasmid pJG2. For experiments involving amber mutants, a streptomycin-resistant derivative of host strain KL16-99 was used to silence a weak amber suppressing activity in the parental KL16-99 (KARAM and O'DONNELL 1973) (see MATERIALS AND METHODS).

As in the experiments reported above, large amounts of replicated (*Hae*III-resistant) conversion products were generated after infection with the control K10 phage (Figure 6A, lanes 3 and 4). In addition, I-*Tev*I cleavage products were detected in DNA from the wild-type (and several mutant) infections. The dsb in A bisects the 3921-bp *Ase*I parental DNA fragment into fragments of 3161 bp and 760 bp. (The larger I-*Tev*I cleavage product is indicated by an open arrow in Figure 6A and the smaller product migrated off the gel).

Turning to the gene *46*-mutant infection, two important observations are apparent. First, the coupled repair/replication reaction is absolutely dependent on gp46 (Figure 6A, compare lanes 4 and 6). Second, the products of I-*Tev*I cleavage are stabilized by the absence of gp46 (Figure 6A, lane 5), consistent with prior studies on the role of gp46/47 in degradation of broken DNA (KUTTER and WIBERG 1968; ALBRIGHT and GEIDUSCHEK 1983; KREUZER *et al.* 1995). Since dsb's are most stable

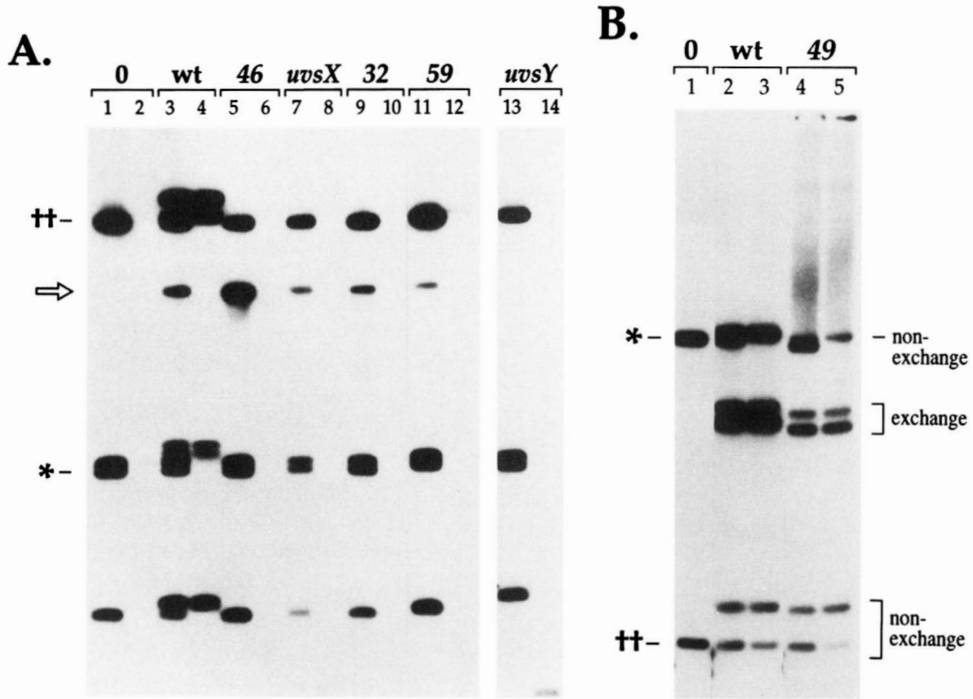

FIGURE 6.—Protein requirements. DNA samples were prepared from either pJG2-containing cells (A) or pJG6-containing cells (B), either without infection (0) or after 40 min of infection with T4 K10 (wt) or the indicated single mutant of T4 K10. In A, odd-numbered lanes contain *Ase*I-digested DNA and even-numbered lanes contain *Ase*I/*Hae*III double digests. The *uvsY* deletion-mutant infection was performed in KL 16-99 host cells, while all other infections were performed in JG99S. The recipient and donor fragments (see text) are indicated by †† and *, respectively, and the I-*Tev*I-cleaved substrate fragment by an open arrow. In B, lanes 1, 2 and 4 contain *Pac*I-digested DNA, and lanes 3 and 5 contain *Pac*I/*Hae*III double digests. The donor and recipient fragments are indicated by †† and *, respectively, and the positions of exchange and nonexchange products are indicated by brackets (see Figure 4A and its legend for expected product sizes). In both A and B, internal size markers are provided by the digest of plasmid DNA from uninfected cells (3921, 1834, 1789, and 1157 bp in A and 6513 and 2219 bp in B).

in the *46*-mutant background, a good estimate of *in vivo* I-*Tev*I activity can be obtained. Approximately 50% of the plasmid DNA was cleaved, indicating that in wild-type infections most of the cleaved plasmid DNA is either degraded or repaired.

Infections lacking UvsX, UvsY, gp32, or gp59 showed negligible dsb repair/replication activity (Figure 6A, lanes 7–14), demonstrating that all four proteins are required for product formation. The I-*Tev*I cleavage product was readily detected from the infections lacking UvsX protein, gp32, or gp59, demonstrating that cleaved substrate was available, but was not detected from the *uvsY*-mutant infection for unknown reasons.

Since ~50% of the repair/replication products have undergone exchange of flanking DNA, it was of interest to determine whether the reaction would proceed normally in the absence of gp49 (endonuclease VII), the T4-encoded enzyme that resolves Holliday junctions and other non-Watson-Crick structures (KEMPER and BROWN 1976; MIZUUCHI *et al.* 1982). Cells harboring plasmid pJG6 (see Figure 4A for diagram of substrate and expected products) were infected with T4 K10-*49* or the control T4 K10. The gp49-deficient infection generated products with and without exchanges of flanking DNA, albeit in substantially reduced amounts (Figure 6B). The reduction in product formation suggests that gp49 might normally play a role in the reaction. However, assuming that the *49*-mutant infection is not leaky, these results imply either that Holliday junction-resolving activity is unnecessary for the repair/replication reaction or that another protein can resolve Holliday junctions in a *49*-mutant infection (see DISCUSSION). Finally, it is interesting to note that some plasmid DNA from the *49*-deficient infection migrated as a smear near the top of the gel. The smear presumably contains unresolved intermediates, perhaps Holliday junctions.

DISCUSSION

A model for the coupled repair/replication reaction: In this study, we have demonstrated that bacteriophage T4 efficiently catalyzes a dsb repair/replication reaction with a plasmid substrate *in vivo*. The introduced dsb induces two conversion products, one with and one without coconversion of a neighboring marker. These conversion events are accompanied by exchange of the flanking DNA ~50% of the time.

One of the most interesting features of our results is that the dsb induces a coupled repair/replication reaction in which the entire plasmid is replicated (*i.e.*, *Hae*III resistant). Using field-inversion gel electrophoresis of packaged DNA, the repaired and replicated plasmid DNA was shown to be in concatemers as long as

packaged T4 DNA (170 kb, or about 20 plasmid monomers) without a single *Hae*III-sensitive (unreplicated) site. Further evidence for tight coupling of repair and replication is provided by our inability to detect repair products in plasmid DNA that has not been fully replicated by T4 (data not shown). In this experiment, we cleaved DNA from a relatively early time of infection (20 min; see Figure 3 for replication kinetics) with restriction enzymes that cleave only unmodified DNA, and we could not detect repair products (repair products in replicated DNA from this time point are easily detectable; see Figure 3). The tight coupling of repair and replication argues strongly that the dsb repair reaction is not following the SZOSTAK *et al.* (1983) model for dsb repair. In this model, replication is confined to the homologous DNA surrounding the dsb because the invasion of both ends of the break are concerted (see Figure 1).

The extensive replication observed in our experiments suggests that the two ends of the dsb do not operate in a concerted fashion. The "half-crossover" model (see KOBAYASHI 1992) proposes that dsb's can be repaired by a mechanism involving only one end of the dsb at a time, although this model does not predict the extensive replication that we observed. Interestingly, MOSIG (1983) proposed a model for the initiation of rdr in T4 that can be adapted for the dsb-induced coupled repair/replication reaction described in this work (see SKALKA 1974 for related model with phage λ). In the MOSIG (1983) model, T4 genomic replication is initiated when a 3′ protruding end of a T4 chromosome invades the homologous region of another T4 chromosome to form a D-loop. Leading-strand replication then commences from the invading 3′ end, with lagging-strand synthesis initiating on the displaced strand of the D-loop.

Based on the MOSIG (1983) model for T4 rdr, we have formulated an alternative model that explains all the features of the coupled dsb repair/replication reaction. To acknowledge the extensive involvement of DNA replication, we refer to the general model as the ECR (extensive chromosome replication) model for dsb repair. We will first discuss the ECR model with reference to the inverted-repeat plasmid substrate analyzed in this study (Figure 7) and then describe how the model might explain the repair of dsb's in nonrepeated DNA (*e.g.*, during intron mobility; Figure 8).

In the context of the inverted-repeat plasmid, we surmise that the repair/replication event is intramolecular. There are two reasons for favoring an intramolecular model with the inverted-repeat plasmid: (1) extensive repair/replication was not detected with a plasmid that sustains a dsb but has no internal repeat (unpublished data), and (2) it seems likely that a search for homology would favor an intramolecular target over an intermolecular target. Nevertheless, we admit that definitive results for an intramolecular reaction are lacking. In any case, the model for replication of the inverted-repeat plasmid (Figure 7) can be modified into an intermolecular model, and an intermolecular ECR model for dsb repair of nonrepeating DNA is discussed below.

We propose that the first step after delivery of the dsb is resection by a 5′ to 3′ exonuclease (Figure 7A, step i; see below for discussion of the role of gp46/47 in resection). Depending on the extent of degradation on the right side of the break, coconversion of the nearby B site may occur. Based on *in vitro* studies of recombination proteins (HARRIS and GRIFFITH 1989; KODADEK *et al.* 1989; YONESAKI and MINAGAWA 1989), we believe that gp32, UvsX and UvsY collaborate in the strand-invasion reaction (step ii). We propose that only one of the two ends invades at any one time, resulting in a single intramolecular D-loop. This D-loop then serves to initiate a replication fork in one direction (leftward in Figure 7; step iii and iv). Replication presumably involves the cohort of proteins that function at the T4 replication fork (gp32, 41, 43, 44, 45, 61 and 62) (reviewed in NOSSAL 1994), along with gp59, which facilitates loading of the T4 helicase/primase complex (BARRY and ALBERTS 1994; MORRICAL *et al.* 1994). After the replication step, branch migration and the appropriate ligation event can result in a lariat that contains a Holliday junction. The junction could potentially be resolved by gp49 (endonuclease VII) or a similar activity to produce either exchange (NS; north-south) or nonexchange (EW; east-west) for the rightward flanking DNA (step v; see also below). This repaired and partially replicated plasmid is a linear product of greater than unit length, with two ends that are homologous to internal regions of the duplex.

Because the product of the first reaction cycle has this unique linear structure with invasive ends, subsequent intramolecular reaction cycles are possible (Figure 7B). If one of the ends invades an inverted repeat within the linear molecule, the cycle can repeat, perhaps indefinitely, producing very long linear concatemers of repaired and replicated plasmid (Figure 7B, step i). This repeating cycle is somewhat reminiscent of the "snapback" model of *in vitro* DNA replication, whereby DNA synthesis occurs by a repeated series of UvsX-catalyzed intramolecular strand-invasion steps (MORRICAL *et al.* 1991).

Alternative outcomes are possible if the second or a subsequent cycle involves strand invasion into a direct, rather than inverted, repeat. Most notably, if strand invasion creates a Holliday junction, which is then resolved as an exchange for flanking DNA, a rolling-circle intermediate could be formed (Figure 7B, steps iii and iv, NS resolution). If the Holliday junction is resolved as a nonexchange, it is possible to generate a "reverse rolling circle" (Figure 7B, steps iii and iv, EW resolution). The leading strand of replication on such a reverse rolling circle is on the tail of the lariat, and the template for that leading-strand synthesis is the lagging-strand product from the previous round of replication.

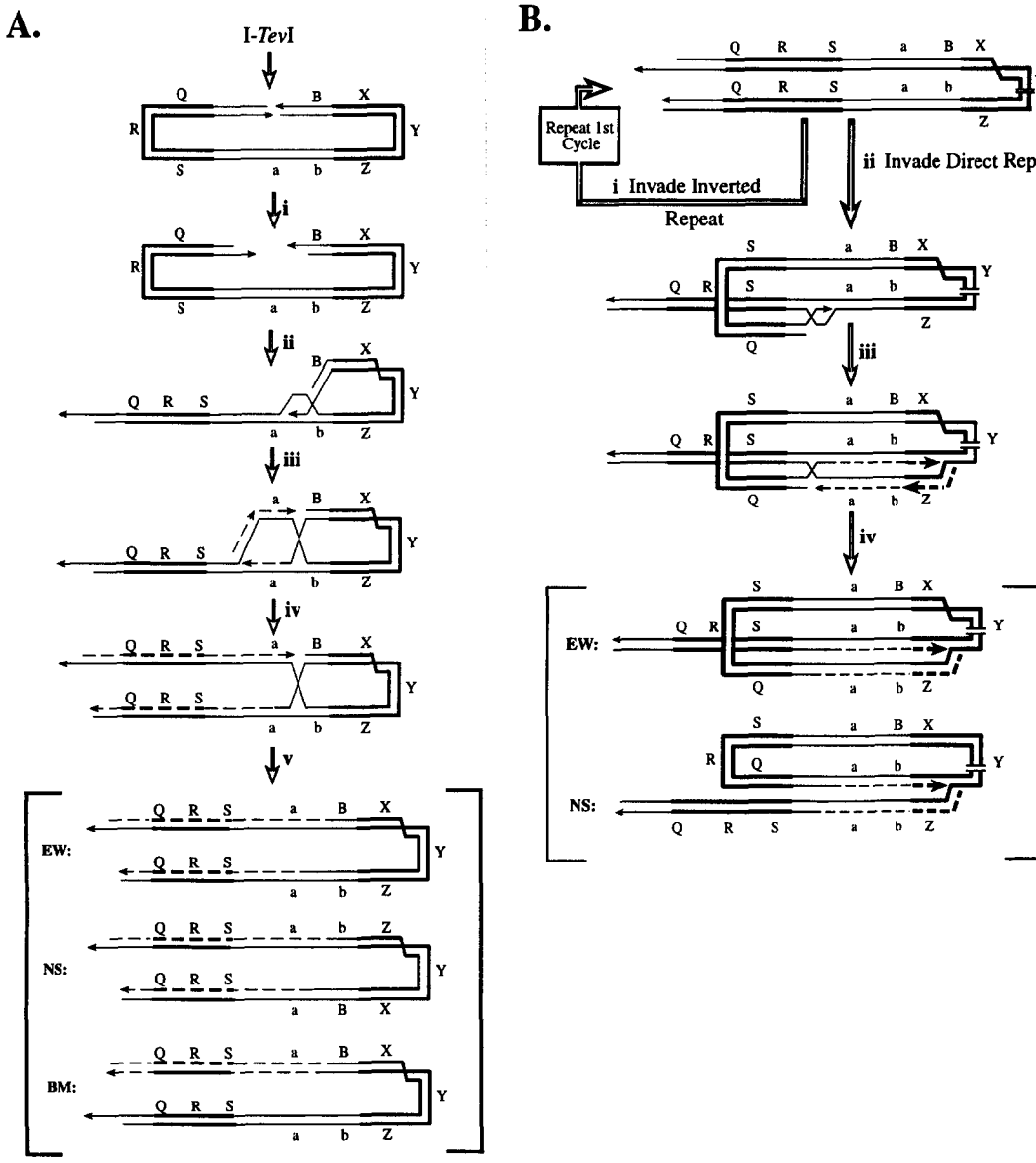

FIGURE 7.—An ECR model for the dsb-induced coupled repair/replication reaction. In step *v* of A, the orientation of Holliday junction cleavage is indicated as east-west (EW) or north-south (NS); as an alternative, resolution may occur by branch migration (BM). In B, the double break near Y indicates the possibility of additional plasmid monomeric units generated by repeated cycles of inverted repeat invasion.

At present, we cannot tell whether the dsb repair/replication reaction occurs by repeated cycles of invasion of inverted repeats, rolling (and/or reverse rolling) circles generated by invasion of direct repeats, or both. Any combination of these pathways could explain the generation of long concatemeric products. Restriction enzyme analysis indicated that adjacent monomeric units of the concatemer are equally likely to have the same or opposite configuration with respect to the exchange of flanking DNA (see Figure 5). This apparently random arrangement of neighbors can be readily explained by the model in Figure 7 in two different ways: (1) repeated cycles of inverted repeat invasion with random choice of EW or NS resolution, and (2) two or more cycles of inverted repeat invasion with random choice of EW or NS resolution, followed by one cycle of direct repeat invasion that leads to a rolling or reverse rolling circle (in which the circle is a dimer or higher multimer).

The ECR model for intermolecular dsb repair: Because our experiments utilized a contrived inverted-repeat plasmid, it is fair to ask whether our results have any relevance to the normal DNA metabolism of T4 (which has no sizable inverted repeats in its genome). We believe the results are indeed relevant to normal T4 DNA metabolism. As described above, the ECR model is really just an adaptation of the MOSIG (1983) model for T4 rdr to the repair of discrete dsb's. Viewed in this context, the inverted-repeat plasmid provides a useful model system for analyzing the molecular details of T4 rdr.

Could the ECR model also explain intermolecular dsb repair in T4, for example, the process of intron

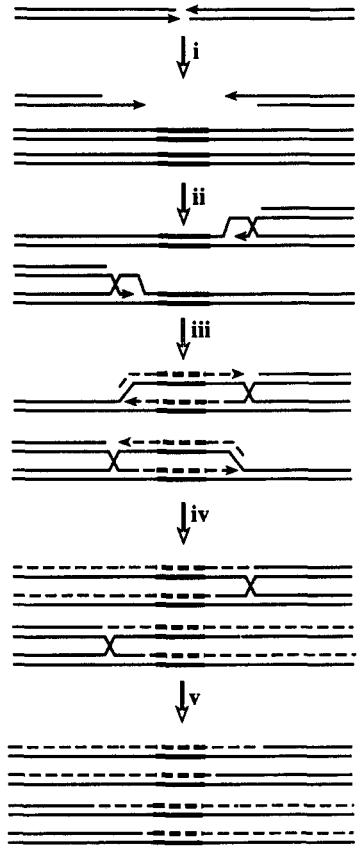

FIGURE 8.—The ECR model for dsb repair. The process of intron mobility is shown as an illustration of intermolecular dsb repair.

mobility or the repair of radiation-induced breaks? The simplest adaptation of the model to an intermolecular reaction would have the two broken ends invading different intact homologues, triggering replication of each. As an example, the process of intron mobility by means of ECR is diagrammed in Figure 8. Invasion of the two broken ends of the intron-free (I-*Tev*I-cleaved) DNA into two different intron-containing homologues results in the establishment of a new replication fork on each homologue. Extensive chromosomal replication follows, along with resolution of the Holliday junctions. The net result is that one cleaved intron-free DNA plus two intron-containing molecules are converted into four intron-containing products.

The ECR model makes sense within the life cycle of a bacterial virus. T4-infected cells have multiple copies of the phage genome, and there is no apparent need for the two broken ends to invade the same donor chromosome. It would seem quite natural for T4 to simply initiate replication every time it comes across a duplex end, rather than coordinate a repair event involving concerted strand invasions and localized DNA replication. Viewed in this context, the mechanics of achieving localized replication and concerted strand invasions in models such as that of SZOSTAK *et al.* (1983) appear formidable.

MUELLER *et al.* (1996) recently analyzed intron mobility in phage T4 using an intermolecular assay in which an intronless plasmid DNA (recipient) sustains a dsb, which is then repaired using an intron-containing phage DNA (donor). As in our studies, they obtained evidence that dsb repair is closely related to rdr, and that the SZOSTAK *et al.* (1983) model is not sufficient to explain all dsb repair in phage T4. However, based on their results, MUELLER *et al.* (1996) proposed that T4 dsb repair can occur by a very different model than the one we favor. In their model, termed SDSA (synthesis-dependent strand annealing), localized replication is achieved by a process of bubble migration (FORMOSA and ALBERTS 1986), in which only one strand of product is generated by a process that resembles transcription. Once synthesis has crossed the intron, the single-stranded product anneals to the exposed 3′ end on the opposite side of the break (hence the name SDSA). The SDSA model clearly cannot account for our results, because it predicts only localized DNA replication. The one key difference in experimental results that led to the two different models is that MUELLER *et al.* (1996) detected an excess of products that had not undergone exchange for outside markers, while we detected an equal ratio of exchange and nonexchange products. What could account for the discrepancy between these two studies? One general possibility is that some difference in experimental conditions (*e.g.*, bacterial host) or setup (*e.g.*, intermolecular *vs.* intramolecular reaction) led to different ratios of products. A more specific possibility is that, in the intermolecular assay of MUELLER *et al.* (1996), nonexchange products are preferentially amplified because they are free circles that undergo rolling-circle replication by both origin- and recombination-dependent modes (the exchange products are plasmid-phage recombinants). Further experiments will be required to determine which (if either) of the ECR or SDSA models accurately reflect the process of dsb repair in phage T4.

The role of gp46/47: gp46/47 is a key recombination protein of T4 but has not yet been characterized biochemically. Mutational inactivation of the protein greatly stabilizes broken DNA *in vivo*, consistent with the proposal that gp46/47 is an exonuclease (KUTTER and WIBERG 1968; ALBRIGHT and GEIDUSCHEK 1983; KREUZER *et al.* 1995). The repair/replication reaction analyzed in this study is totally dependent on gp46, which could be explained if the putative exonuclease activity is necessary for exposing 3′ single-stranded ends at the dsb within the plasmid substrate. In contrast, gp46/47 is not strictly required for rdr of plasmids with homology to the T4 genome, whether or not a dsb is introduced into the homologous region of the phage chromosome (KREUZER *et al.* 1988b, 1995). One interesting possibility is that single-stranded 3′ ends at a T4 chromosomal dsb can be provided by origin-dependent replication, because of the difficulty of replicating the 3′ ends of a linear DNA. In the plasmid-based system reported here, the broken substrate lacks a T4 origin

of replication and would thus require activation by an exonuclease.

If gp46/47 is the proposed 5′ to 3′ exonuclease required for rdr and dsb repair, it might be a multifunctional protein with exonuclease, NTPase and DNA helicase activities similar to the RecBCD enzyme of *E. coli* (reviewed in KOWALCZYKOWSKI *et al.* 1994; MYERS and STAHL 1994). Consistent with this notion, gp46 contains a good match to the expanded "Walker A site" consensus for ATP binding (GORBALENYA and KOONIN 1990).

The role of gp49 and exchange of flanking DNA: The resolution of the Holliday junction is a critical step in the ECR model discussed above. With regard to the repair/replication reaction with the inverted-repeat plasmid, resolution is necessary to generate rolling (or reverse rolling) circles and might seem to be necessary for the observed exchange of flanking DNA (Figures 4 and 6B). We therefore expected that endonuclease VII (gp49) would be absolutely required. However, although a gene *49* mutation reduced the extent of the reaction by a modest amount, the residual reaction still produced exchanges and nonexchanges for flanking DNA with roughly equal efficiencies (also see MUELLER *et al.* 1996). It is possible that the residual dsb repair/replication reaction is dependent on a low level of endonuclease VII activity in the amber mutant. However, we obtained essentially identical results at nonpermissive temperature with the gene *49* temperature-sensitive allele *tsC9* (data not shown), which has the most severe phenotype of *49* mutations (G. MOSIG, personal communication). The incomplete dependence on gp49 is consistent with two possibilities. First, an alternative Holliday junction-resolving nuclease may be active in T4-infected cells. This putative activity could be either of the host-encoded resolving nucleases (RuvC or Rus; WEST 1994; SHARPLES *et al.* 1994) or an unrecognized protein encoded by either T4 or *E. coli*. Second, the dsb repair/replication reaction may not require a Holliday junction-resolving nuclease, in spite of the generation of exchanges. For example, the model in Figure 7 suggests a simple possibility. After DNA replication has produced a complete lariat, the Holliday junction could be resolved by invoking leftward branch migration all the way to the ends of the broken molecule (Figure 7, step v, BM). The resulting molecule would contain both an exchange and a nonexchange, explaining the generation of exchanges without cleavage of the Holliday junction.

Repair and replication in other systems: Very similar dsb-triggered replication models might account for dsb repair and related events in the following diverse situations. First, the SOS system of *E. coli* results in the process of inducible stable DNA replication (reviewed in ASAI and KOGOMA 1994), which can initiate extensive DNA replication from a dsb (ASAI *et al.* 1994). Inducible stable DNA replication can be viewed as a repair system for the dsb's that originally induced the SOS response. Second, a recent model for recombination during the processes of conjugation and generalized transduction in *E. coli* involves replication of the entire bacterial chromosome initiated from the two invading ends of the incoming fragment (SMITH 1991; ASAI *et al.* 1994). Third, the phenomenon of group 1 intron mobility has been documented not only in the T-even phages, but also in the mitochondrial DNA of lower eukaryotes (reviewed in LAMBOWITZ and BELFORT 1993). Particularly because mitochondrial DNA is multicopy, it is tempting to speculate that intron mobility in mitochondria involves a coupled repair/replication reaction like the one analyzed here. Fourth, Haber's group has recently obtained evidence indicating that yeast chromosomal dsb's can be repaired by a pathway that involves replication from the site of the break to the end of the chromosome (J. HABER, personal communication). In these experiments, break repair in a diploid cell was accompanied by homozygosis of all tested markers from the site of the break to the telomere, arguing that an intact chromosome was regenerated by replication using the homologue as template. In these and perhaps many other situations, dsb-triggered genomic replication could be an effective mechanism to repair breaks. In this context, most genetic studies of dsb repair are blind to the extent of DNA replication, and so coupled repair/replication reactions may be more prevalent than commonly assumed.

The experiments in this report have provided a new and useful method for measuring dsb repair and replication in bacteriophage T4. The inverted-repeat plasmid provides a self-contained substrate to analyze a site-specific version of T4 rdr without the complication of concurrent origin-dependent replication that occurs on the phage genome. Most notably, the results indicate that extensive DNA replication is an inherent part of the dsb repair process, and they provide further support for the notion that rdr and recombination-mediated repair are mechanistically identical in T4. The results support a model in which dsb repair is accomplished without coordinating the invasion of the two broken ends, and this ECR model could explain dsb repair in diverse systems.

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