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Test of the Double-Strand-Break Repair Model of Recombination in *Xenopus laevis* Oocytes

SUNJOO JEONG-YU† AND DANA CARROLL*

Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84132

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A direct test was made of predictions of the double-strand-break repair (DSBR) model of recombination in *Xenopus laevis* oocytes. The DNA substrate injected into oocytes had two directly repeated copies of a 1.25-kb sequence and was cleaved within one of them. Different products were expected to result from concerted, conservative events, as predicted by the DSBR model, and from nonconservative events. Only very low levels of recombination products, both conservative and nonconservative, were observed. When individual, apparent DSBR products were cloned and characterized, it emerged that the majority of them had arisen by nonconservative recombination through short, terminal homologies and not from the gene conversion events predicted for DSBR. Two cloned products among 44 tested corresponded to the predications of the DSBR model, but these could also have been generated by other processes. The most efficient recombination events in oocytes are nonconservative and are based on long, terminal homologous overlaps; when these are not available, short, imperfect overlaps support a lower level of nonconservative recombination; genuine, conservative DSBR events occur rarely, if at all.

In addition to its crucial role in meiosis, homologous genetic recombination has been observed in somatic cells of a variety of organisms. Perhaps the best-known example is the yeast *Saccharomyces cerevisiae*, in which double-strand breaks (DSBs) in chromosomal or plasmid DNA are repaired in a homology-dependent fashion (24, 28). Linear DNA molecules introduced into yeast cells also become substrates for this repair system (25). When the exogenous DNA shares sequences with a chromosomal site, the two recombine relatively efficiently; this recombination allows targeted manipulations of the yeast genome that are not as feasible in most other organisms.

On the basis of the properties of this transformation process in yeast cells, Szostak et al. (38) proposed a molecular model, which is also able to account for some features of meiotic recombination in yeast and other fungi. This DSB repair (DSBR) model envisions that both ends created by a single break interact coordinately with an intact copy of homologous sequences on the same or another chromosome. The break is repaired by localized DNA synthesis using the intact copy as a template, and resolution of the recombination junction may occur either with or without concomitant exchange of flanking markers. This process is ultimately conservative, in the sense that both the broken and the intact copy of the interacting DNA sequences are retained.

Several investigators have tested whether a similar recombination mechanism is operative in other species. This is of interest both with respect to the generality of recombination mechanisms and from the point of view of engineering genetic alterations in other organisms. While the repair of DSBs and gaps has been demonstrated in other eukaryotic cell types (4, 12, 15), they are generally not able to support DSBR events with the efficiency seen in *S. cerevisiae* (2, 9, 14, 33, 36). The rule seems to be that nonconservative recombination predominates in these systems.

Recombination of DNAs introduced into *Xenopus* oocytes is very efficient and completely homology dependent (6). Characterization of recombination intermediates has provided strong support for a nonconservative, annealing pathway in oocytes (23). The basic features of this mechanism are that double-strand ends are resected by a 5′→3′ exonuclease (22) until complementary sequences are exposed on 3′-terminated single strands; these sequences anneal, forming an association that is matured by continuing exonuclease degradation until the redundant sequences are completely assimilated (23); DNA ligase joins the parental strands, possibly after some participation by DNA polymerase.

In our previous studies, we used substrates that would not readily have shown conservative recombination products. Here we examine directly the ability of oocytes to accomplish DSBR-type recombination by using a substrate that carries a broken and an intact copy of a duplicated sequence on the same molecule. Similar designs have been employed in other systems (9, 31). We found that oocytes generate few, if any, genuine DSBR products. When the efficient, nonconservative recombination pathway is blocked, a less efficient but still nonconservative process that uses short, imperfect homologies is responsible for most of the low level of products recovered.

MATERIALS AND METHODS

Plasmid pDC10 carries 1,691 bp of adenovirus type 2 (Ad2) DNA (coordinates 22233 to 23924) between 1,246-bp direct repeats of the tetracycline resistance (Tet) gene of pBR322 (Fig. 1) (6). The Ad2 insert is flanked by a *Kpn*I site on one end and a *Xho*I site on the other. One copy of the Tet repeat has eight sites that have been modified by single-base-pair substitutions to create or destroy individual restriction enzyme sites (15a). Plasmid pDMI,1 (8) has no homology to pDC10; it was used in some experiments to keep the total DNA concentration constant while pDC10 was diluted.

* Corresponding author.
† Present address: Department of Developmental Biology, Beckman Center, Stanford University Medical School, Stanford, CA 94305-5427.

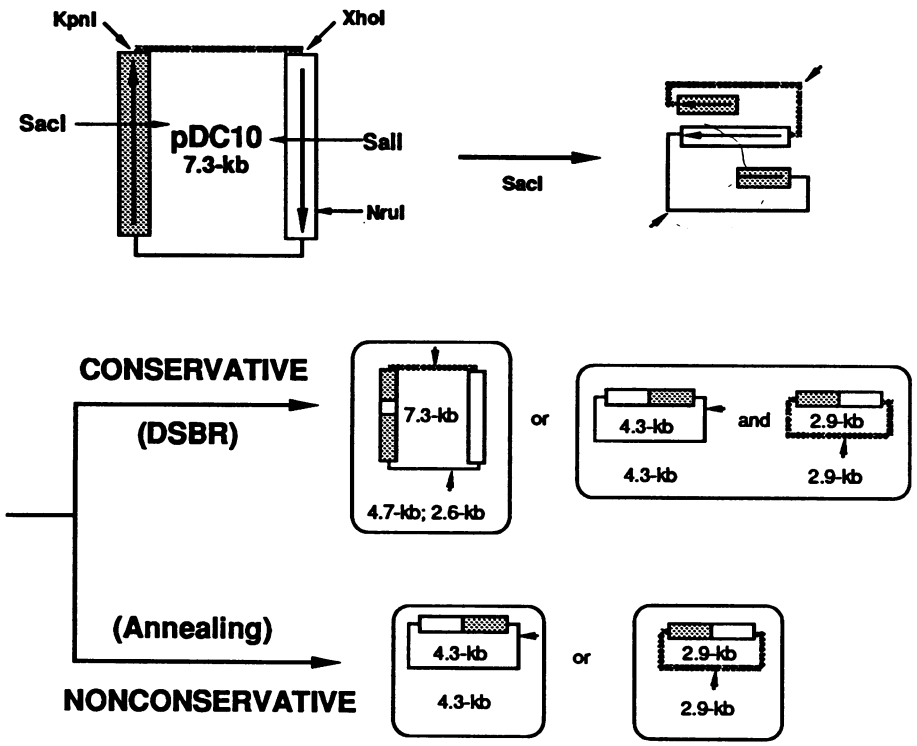

FIG. 1. Diagram of the substrate and expected recombination products. The plasmid used in these experiments was pDC10; it consists of pBR322 sequences (the open box represents the Tet gene, and the thin line represents the remainder of pBR322), a 1,246-bp duplication of the Tet gene in which eight single-base-pair substitutions have been made (shaded box), and 1,691 bp of Ad2 DNA (textured line). The Tet repeats are in direct orientation, as indicated by the arrows. Cleavage at the unique *Sac*I site in one of the Tet repeats generates a molecule that can undergo either conservative recombination events, as predicted by the DSBR model, or nonconservative events, as predicted by the annealing model. The expected products for each of these types of events are illustrated; the sizes of fragments generated by *Pvu*II digestion of each product are also given. The locations of *Pvu*II sites are shown with small, unlabelled arrows on the substrate and product diagrams. Sites for enzymes used to generate substrates for injection are shown on the pDC10 diagram.

Circular pHSS6 DNA (34), which has very little homology to pDC10, was coinjected as an internal control for DNA recovery.

Methods for preparing DNA and oocytes, performing injections, recovering DNA, and analyzing it by blot hybridization or bacterial transformation have been described elsewhere (6, 22). Typically, 10 ng of DNA was injected into the nucleus of each oocyte and incubated overnight. DNA was recovered from manually dissected nuclei, digested with restriction enzyme(s), subjected to electrophoresis, transferred to a ZetaProbe nylon membrane (Bio-Rad Laboratories, Richmond, Calif.), and hybridized with a random-primed pBR322 probe. Bacterial hosts for transformation were C600 and DH1 (20). Miniprep DNAs were isolated from individual colonies (20) and analyzed by restriction enzyme digestion. For sequencing of double-stranded DNA, plasmids were isolated from medium-scale (20- to 80-ml) cultures by alkaline lysis and purified by banding in one ethidium bromide-cesium chloride gradient (20).

RESULTS

Experimental design. Plasmid pDC10 (Fig. 1) contains two directly repeated copies of the Tet gene of pBR322 (boxes in Fig. 1), separated on one side by 3.1 kb of pBR322 sequences and on the other by 1.7 kb of Ad2 DNA. Its overall size is 7.3 kb. The two Tet repeats differ from each other at eight restriction sites. In particular, sites for *Sal*I and *Nru*I have been removed from the mutant copy (shaded box), and a new *Sac*I site has been created. Cleavage at a unique site in one repeat, e.g., *Sac*I, generates the substrate that we have injected into oocyte nuclei.

The DSBR model (38) predicts that the resulting terminal repeats will engage in concerted recombination events to yield products that preserve essentially all parental sequences ("conservative" in Fig. 1). When the two ends interact with the intact Tet gene, they will repair the break by copying sequences from the intact copy. Resolution of the predicted intermediate can occur without crossing over, yielding a reconstructed 7.3-kb plasmid, or with crossing over, generating two smaller plasmids, 4.3 and 2.9 kb, from a single event. Because both ends at the original DSB interact simultaneously with a single homology, these are sometimes called two-ended events. The unidirectional transfer of information at the repair site results in a local gene conversion. Thus, these events are locally nonreciprocal, but the exchange of flanking markers is generally reciprocal. The terms "gene conversion" and "reciprocal recombination" have both been used to describe some features of DSBR-like events.

Alternative recombination pathways envision the interaction of only one homologous end with the intact Tet gene. A single, one-ended event of this type would generate one small plasmid and delete sequences between the site of recombination and the noninteracting end ("nonconservative" in Fig. 1). Either type of small plasmid, 4.3 kb or 2.9

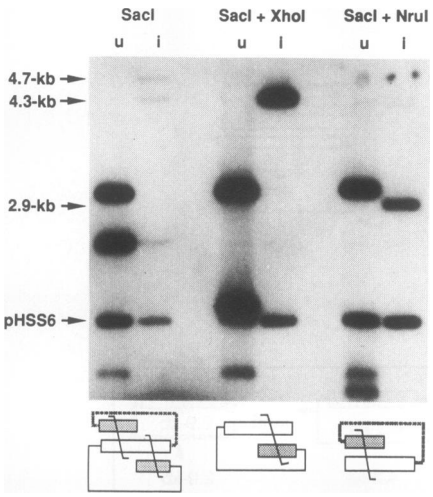

FIG. 2. Results of injection of a potential DSBR substrate. pDC10 was digested with the indicated enzyme(s), injected into oocytes, incubated overnight, recovered, and digested with *Pvu*II for analysis. After electrophoresis in 1% agarose, the DNA was transferred to a nylon membrane and hybridized with a pBR322 probe. Uninjected (u) and injected (i) samples are shown for each DNA substrate. Diagrams of the substrate structures and potential recombination events are shown below the corresponding lanes. The *Sac*I-digested DNA is the DSBR substrate; it yielded two anticipated recombination products, the 4.7-kb band diagnostic of reconstruction of the 7.3-kb plasmid and the 4.3-kb band that could come from conservative or nonconservative events, in very low amounts. The two control DNAs having simple terminal homologies were pDC10/*Sac*I + *Xho*I, which yielded the expected 4.3-kb recombinant in large amounts, and pDC10/*Sac*I + *Nru*I, which yielded the 2.9-kb recombinant with somewhat lesser efficiency. Circular pHSS6 was coinjected as a control for recovery.

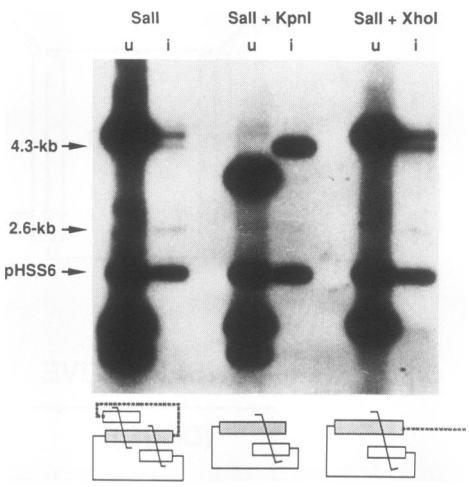

FIG. 3. Comparison of the DSBR substrate with a simple end-blocked substrate. In this experiment, the DSBR substrate was prepared by cleaving pDC10 with *Sal*I, which cuts in the wild-type copy of the Tet repeat (see Fig. 1). As with pDC10/*Sac*I, it yielded very low levels of recombination products; in this case, the 2.6-kb fragment is the clearest diagnostic of reconstruction of the 7.3-kb plasmid, since a *Pvu*II fragment at 4.7 kb can also be generated from substrate that has not completely disappeared after overnight incubation. pDC10/*Sal*I + *Kpn*I provided the control for efficient recombination; pDC10/*Sal*I + *Xho*I has a simple nonhomology on one end. The expected recombination product in both these cases is the 4.3-kb band. Structures of the substrates are diagrammed below the appropriate lanes. Experimental details are as for Fig. 2.

kb, could be produced, depending on which end engaged in recombination, but only one plasmid would result from each event. One example of a nonconservative mechanism is the single-strand annealing model (7, 17, 23).

Our strategy was to inject pDC10 that had been cleaved within one of the Tet homologies, allow recombination in the oocytes, then analyze specifically for the production of the 7.3-kb plasmid. Figure 1 shows the restriction sites used for analysis of the recovered DNAs and the fragments expected from the various products. The diagnostic fragments are the same for intramolecular events, as illustrated in Fig. 1, and for intermolecular events, which are also expected to occur. It should be pointed out that multiple, intermolecular, nonconservative events can generate products that are indistinguishable from those that would result from concerted, conservative events (see Discussion).

Recombination efficiency. The results of a typical recombination experiment are shown in Fig. 2. *Sac*I-digested pDC10 has terminal Tet homologies of about 0.8 and 0.4 kb; it was injected into oocytes, incubated overnight, recovered, and analyzed by digestion with *Pvu*II. Very faint bands can be seen at 4.7 and 4.3 kb, representing expected recombination products. Linear DNA is degraded in oocytes (22, 44), so little or no unrecombined substrate was recovered after overnight incubation; only recombination products and circular pHSS6 were seen. The 4.7-kb band is diagnostic for products of DSBR-like events. The 4.3-kb products can be produced by either conservative or nonconservative events. In the former case, an equal yield of 2.9-kb products is expected, but was not observed, even on longer exposure of autoradiograms like that in Fig. 2.

Two control substrates were used to show that the oocytes were capable of efficient homologous recombination: pDC10 digested with *Sac*I plus *Xho*I or with *Sac*I plus *Nru*I. These digests produce molecules that can recombine through terminal homologies of 0.8 kb to produce the 4.3-kb plasmid or of 0.15 kb to produce the 2.9-kb plasmid, respectively. Both controls gave the expected products in good yields (Fig. 2). Thus, the oocytes recombined terminal homologies efficiently, as we have seen repeatedly (6, 22, 23), while the internal homology of the DSBR substrate was utilized very inefficiently.

If nonconservative events predominate in oocytes, the reason for the low yields with pDC10/*Sac*I may be that when one end engages in recombination, the other end interferes by behaving as a terminal nonhomology. We have found that simple nonhomologies greatly reduce the yield of recombinants; intermediates are formed, but their resolution is prevented by failure to remove the nonhomology (13a). When we compared the recombination of the DSBR substrate to one with a simple terminal nonhomology, we found that they yielded very similar levels of 4.3-kb products (Fig. 3), supporting the notion that they arise largely by nonconservative events. In this experiment, the DSBR substrate was made by cleaving pDC10 in the other Tet gene with *Sal*I (Fig. 1), which leaves about 0.6 kb of homology at each end. The fact that low yields of potentially conservative products were again observed demonstrates that the earlier results were not peculiar to the *Sac*I-digested substrate. The substrate with a simple terminal nonhomology was made by digesting pDC10 with *Sal*I plus *Xho*I, which removes one of the terminal Tet homologies. Only small amounts of 4.3-kb

TABLE 1. Cloned recombination products[a]

DNA	No. of clones analyzed	No. with indicated structure				
		7.3 kb, *Sac*I$^-$			7.3 kb, *Sac*I$^+$	4.3 kb
		Large deletion	Small deletion	Gene conversion		
Circular pDC10	7[b]	0	0	0	7	0
Uninjected pDC10/*Sac*I	19	1	0	0	17	1
Injected pDC10/*Sac*I	62	6	31	2	5	18

[a] Individual cloned recombination products were categorized as follows. First, the size of the intact plasmid was determined by electrophoresis of uncut DNA. Digestion with *Sac*I showed whether that site had been regenerated in the 7.3-kb products. Large deletions (>100 bp) were detected by digestion with *Eco*RI plus *Bgl*II. Small deletions (<40 bp) were analyzed by electrophoresis of *Nae*I digests in 8% polyacrylamide gels; several of these were sequenced (see Fig. 5). Gene conversions were *Sac*I$^-$ but showed no deletion; they were confirmed by sequence analysis (see Fig. 4).

[b] Many more pDC10 transformants have been analyzed in other experiments, with the same outcome, but only seven were examined in parallel with the other DNAs in the experiments reported here.

product were generated from this substrate in oocytes. As before, a substrate with homologous sequences on both ends (pDC10/*Sal*I + *Kpn*I) showed efficient recombination, as reflected in the yield of the 4.3-kb product.

It is possible that concerted interaction of the two ends of one substrate molecule with a single internal homology was prevented by the high DNA concentrations used in these injection experiments. Essentially, the ends would be diverted to different molecules, and all apparent conservative products would have to arise by multiple intermolecular interactions. We tested whether conservative events would be increased in conditions that favor intramolecular processes by injecting pDC10/*Sac*I at lower concentrations. The total DNA concentration was maintained at 10 ng per oocyte by addition of a nonhomologous linear DNA (pDMI,1/*Hin*dIII), while the substrate concentration was lowered to 2 and 0.5 ng per oocyte. Such dilution with nonhomologous linear DNA has been shown to lead to largely intramolecular recombination (21). We found no increase in the yield of DSBR products in these conditions (data not shown).

In addition, we measured the ratio of intra- and intermolecular events directly by using pairs of marked substrates (13, 21). Even at the highest input of substrate DNA (10 ng per oocyte), about 40% of the recombination products were from intramolecular events (data not shown), indicating that diversion of ends was not a serious problem.

Characterization of recombination products. The foregoing results showed that some apparent DSBR products were formed, although the yield was quite low. To determine whether these products had the structure predicted from genuine DSBR events, we cloned a number of them and determined the structure of the repaired junctions. The DSBR model predicts that the 7.3-kb plasmids will have a complete reconstructed Tet gene that has acquired sequences from the intact copy at the repair site. For example, products from *Sac*I-digested pDC10 would have replaced the mutant sequence (*Sac*I$^+$) with the corresponding wild-type (*Sac*I$^-$) version (Fig. 1). Alternative routes to 7.3-kb products might be the simple rejoining of the *Sac*I ends or recombination based on short, terminal homologies (11).

DNA recovered from oocytes injected with pDC10/*Sac*I was used directly to transform bacteria (Table 1). Circular products of intramolecular recombination events would transform most efficiently and be preferentially recovered in this protocol. Circular pDC10 and uninjected pDC10/*Sac*I were transformed as controls. Plasmid DNAs were isolated from individual colonies and analyzed by gel electrophoresis. Plasmids with an apparent size of 7.3 kb were further analyzed by restriction enzyme digestion.

Among 62 transformants from injected pDC10/*Sac*I, 44 carried 7.3-kb plasmids; the remaining 18 had 4.3-kb plasmids (Table 1). (The 2.9-kb circular product has neither an origin of replication nor an amplicillin resistance gene and thus would not be recovered.) The cloned 7.3-kb products were analyzed by restriction enzyme digestion to provide information about the nature of the recombination junctions (Fig. 4). Five of the plasmids were cleaved by *Sac*I, indicating that they resulted either from a low level of undigested substrate molecules or from religation of the *Sac*I site in oocytes or in bacteria. The 39 remaining 7.3-kb plasmids were *Sac*I$^-$ (Fig. 4A). Digestion of the products with *Eco*RI plus *Bgl*II generates a 2.1-kb fragment that contains the recombination junction (Fig. 4B); six of the 7.3-kb plasmids showed large deletions in this fragment (Table 1). Digestion with *Nae*I produces a 160-bp fragment containing the junction and one of the same size from the wild-type repeat (Fig. 4C). Comparison of these two fragments by polyacrylamide gel electrophoresis showed small deletions at the junctions in 31 of the 7.3-kb plasmids. Only two of the *Sac*I$^-$ plasmids (including the one in lane 6 of Fig. 4C) had *Nae*I fragments of the original size; these are referred to as gene conversion products in Table 1.

All of the control transformants with circular pDC10 carried a 7.3-kb plasmid that was sensitive to *Sac*I, as expected (Table 1). Among plasmids from 19 transformants with uninjected pDC10/*Sac*I, 17 were religated (or undigested) pDC10 (i.e., *Sac*I$^+$), one was a 7.3-kb plasmid with a small deletion at the junction, and one was a 4.3-kb plasmid. These distributions are very different from that seen with the injected DNA, so we can be confident that most of the recombinants in the latter sample were indeed produced in oocytes. In addition, the normalized transformation efficiency of injected pDC10/*Sac*I was 10- to 20-fold higher than that of an uninjected control, indicating that some linear molecules were recyclized in the oocytes.

Sequences of recombination junctions. Nucleotide sequences at the junctions were determined for the two 7.3-kb plasmids with undeleted junctions and for several of those with small deletions. Double-stranded plasmid DNAs were sequenced by extension from a primer that includes the mutant *Nru*I site of the cleaved repeat (shaded in Fig. 1). In preliminary experiments, priming from the wild-type Tet repeat also occurred, even though the 17-nucleotide primer had one mismatch with the corresponding sequence in that repeat. To eliminate sequence ambiguity, each plasmid was cleaved with *Nru*I prior to sequencing, thus interrupting the unwanted primer binding site in the wild-type copy.

Both of the undeleted junctions had the sequence expected for DSBR products (Fig. 5). The *Sac*I sequence (GAGCTC) was replaced by the sequence from the wild-type repeat (GCGCTC), and there were no other alterations. Additional restriction enzyme analyses showed that the nearest distinguishable sites, a *Sal*I site 165 bp to the left and an *Nru*I site 158 bp to the right, were both of the mutant type in these recombinants; therefore, the apparent conversion tract was less than 333 bp.

Sequences of six of the small-deletion junctions are shown in Fig. 6. Each has apparently arisen by homologous recombination between adventitious short repeats near the *Sac*I-generated ends. Four of them occurred in the same 10/13-bp

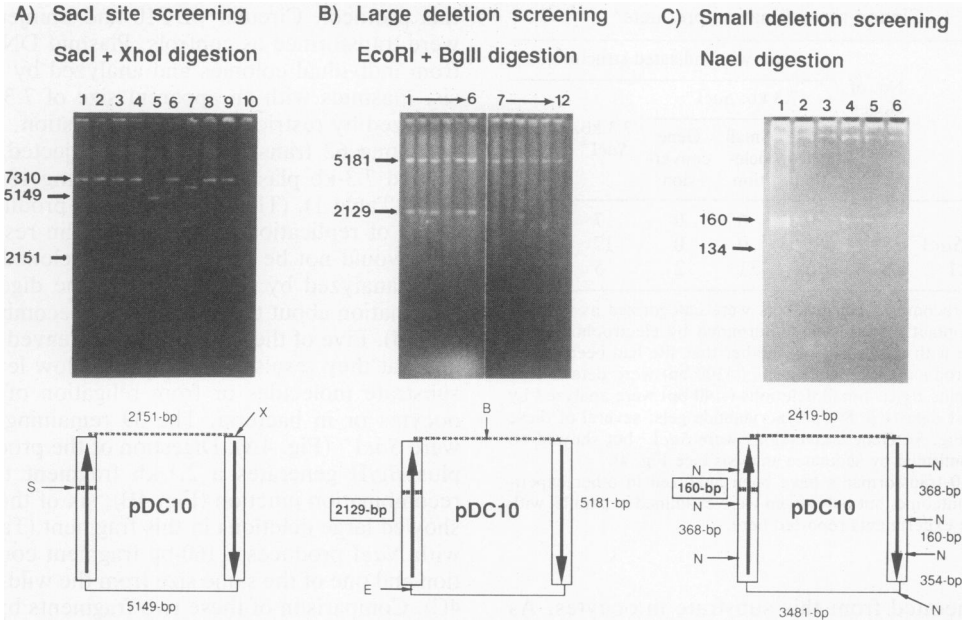

FIG. 4. Examples of restriction enzyme analyses of cloned 7.3-kb recombination products. Plasmid DNAs were isolated from individual colonies after transformation of bacteria to ampicillin resistance, using DNA from oocytes that had been injected with pDC10/*Sac*I. Diagrams showing the locations of the diagnostic restriction sites and the expected fragment sizes are shown below each panel. (A) Digestion with *Xho*I + *Sac*I and electrophoresis in a 1% agarose gel. Most plasmids showed only the 7,310-bp fragment resulting from cleavage by *Xho*I alone (lanes 1 to 4, 6, 8, and 9) and thus lack the *Sac*I site. One plasmid carries a *Sac*I site and gives fragments of 5,149 and 2,151 bp (lane 5). Another (lane 7) has only the *Xho*I site but carries a large deletion that causes it to run considerably faster than the 7,310-bp DNAs. Lane 10 contains a 4.3-kb plasmid that is resistant to digestion by either enzyme and runs as supercoiled and nicked circles. (B) Digestion with *Eco*RI plus *Bgl*II and electrophoresis in a 1.5% agarose gel. The plasmids in lanes 1 to 6 gave fragments indistinguishable from pDC10, while those in lanes 7 to 12 all showed deletions, of various sizes, in the 2,129-bp fragment, which contains the recombination junction. (C) Digestion with *Nae*I and electrophoresis in an 8% polyacrylamide gel. As indicated by the diagram, parental pDC10 yields two 160-bp fragments, one of which contains the *Sac*I site (shaded). The plasmids in lanes 1 to 5 each showed one parental and one deleted fragment in this size range; that in lane 6 showed only the parental size of fragment and is referred to as a gene conversion product. Larger *Nae*I fragments all ran near the top of this gel.

repeat, while one each occurred in matches of 8 of 11 and 5 of 6 bp. These results correspond to our earlier observations of low-level recombination using short terminal overlaps (11). In that study, matches in the range of about 8 to 15 bp yielded rare recombinants, and the efficiency was greater when the match was longer. Only matches within about 20 bp of both molecular ends produced recombinants (11).

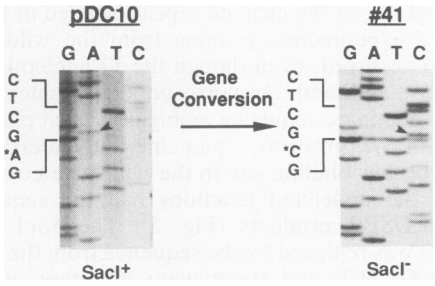

FIG. 5. Nucleotide sequence of a gene conversion product. A portion of a sequencing gel using pDC10 as a template is shown at the left; the *Sac*I site is bracketed, and its sequence is given. On the right is the corresponding region of a sequence analysis of one of the cloned gene conversion products, #41; the sequence of the altered *Sac*I site is bracketed. The A in the pDC10 sequence and the C that has replaced it in the #41 sequence are indicated with arrowheads. In the #41 sequence, the G at the 5′ end of the altered site gave bands in all lanes, but this does not obscure the obvious A-to-C change at the next position.

DISCUSSION

Recombination in oocytes. A direct test of the DSBR pathway for recombination in *Xenopus* oocytes has shown that the conservative events predicted by this model occur very rarely, if at all. A substrate designed explicitly to detect such events yielded very low levels of recombination products. Even among the products that might have been interpreted as arising from DSBR events, the great majority actually arose from nonconservative events. DNA sequence analysis showed that short homologies at the molecular ends were utilized and resulted in small deletions at the junction.

Only 2 among 44 cloned, reconstructed 7.3-kb plasmids had the junction sequence predicted to result from gene conversion as envisioned in the DSBR model. These products may have arisen from genuine DSBR events, through the concerted interaction of both ends generated by the DSB with one copy of the intact Tet homology (Fig. 1). There are, however, at least two alternative possible sources of such products. First, they may have been generated by multiple, intermolecular, nonconservative (one-ended) events; we have no direct evidence that these particular products arose by intramolecular recombination. Second, the ends of the cleaved Tet gene might have recombined with a fragment from the intact copy that could have been generated by a low level of random breaks in the injected DNA. If such fragments exist, they could recombine with the ends generated by intentional *Sac*I cleavage through the very efficient, nonconservative annealing pathway that is known to operate in oocytes (23). The fragments that participated in generating

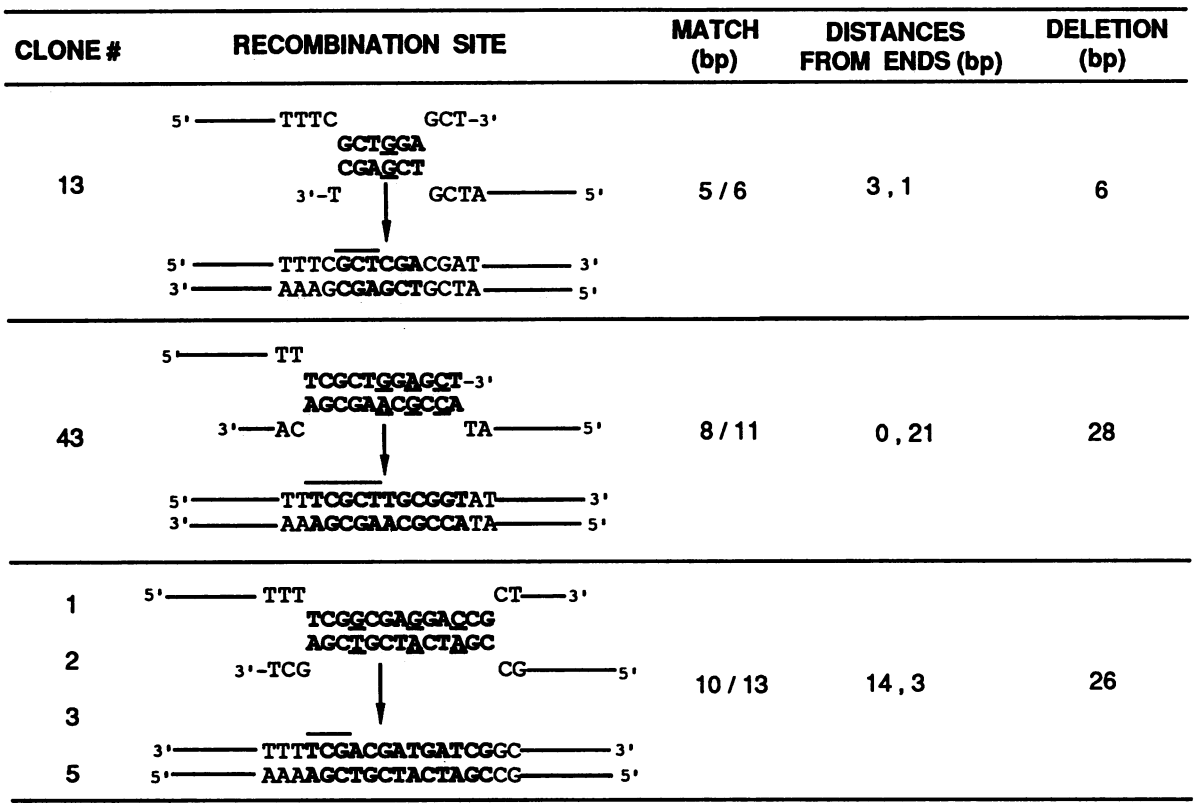

FIG. 6. Nucleotide sequences of recombination products having short deletions. For each clone, the sequence determined for the double-stranded recombination junction is shown on the lower lines. Above that is an interpretation of the annealed homology between the 3'-terminated strands of the parental sequences that is a plausible intermediate in the formation of that recombinant. Bases within the match are in bold type, and mismatches are underlined. Overlines indicate the region of perfect match in which the effective crossover has occurred. Sequences longer than 3 bases outside the matches are shown as lines. Also given are the number of bases in each overlap that are complementary (match), the distance of the matched sequences from the original 3' ends (including the 4-nucleotide 3' protruding ends left by *Sac*I cleavage), and the number of base pairs deleted compared with pDC10. Four independent clones (clones 1, 2, 3, and 5) had the same sequence.

the two sequenced gene conversion products would have to have been rather small, since they contributed no additional sequences beyond about 160 bp on either side of the repaired junction.

The recombination of molecules with long, terminal homologous overlaps is very efficient in oocytes (6). In a long incubation, all recovered molecules are recombinants, and the overall recovery is often in the range of 50 to 100%. The efficiency of recombination using short terminal repeats is much less and varies with the quality of the matches between those repeats (11). In our experiments with the DSBR substrate, the total amount of 7.3-kb plasmid product, as judged by scanning densitometry of the 4.7-kb band in autoradiographs like that in Fig. 2, was about 5% of the yield of 4.3-kb product from the efficient substrate (pDC10/*Sac*I + *Xho*I). Among cloned 7.3-kb recombinants, only 4% (2 of 44) were gene convertants. Thus, the yield of apparent DSBR products was 0.2% of the yield of efficient homologous recombinants. This is an upper limit for the relative efficiency of DSBR in oocytes, since as elaborated above, it is possible that these molecules arose from nonconservative events.

We assume that the 4.3-kb recombination products from the DSBR substrate arose largely or entirely from nonconservative events (Fig. 1). The yield of 2.9-kb recombinants, also expected from similar events using the other homology, was always substantially lower and often undetectable. A plausible explanation is that the effective terminal nonhomology blocking production of the 2.9-kb plasmid was longer (3.9 kb) than that blocking production of the 4.3-kb recombinant (2.1 kb). We have found that longer blocks inhibit recombination more severely (13a).

Comparisons with other organisms. Tests of the DSBR model of recombination have been made in other organisms, sometimes with substrates quite similar to that used here. Two separate investigations of the bacteriophage λ Red pathway have shown that the products predicted by DSBR can, in fact, be identified (39, 41). Nonetheless, most Red-mediated events are nonreciprocal (40), which is also true of events in oocytes. Like oocytes, a 5'→3' exonuclease plays a key role in λ recombination (35). Unlike the case for oocytes, however, it appears that a DSB in only one of the parental DNAs is sufficient to allow efficient recombination in the Red pathway (40).

When linear DNAs are introduced into mammalian cells, they can undergo either homologous recombination or nonhomologous end joining (29, 36). The major homology-dependent process appears to be nonconservative, as in oocytes (36). A specific test of the DSBR model revealed deletion products, rather than gene conversion products, as the principal outcome (9). The results from several laboratories are consistent with an annealing model of recombina-

tion (1, 10, 14, 17–19, 33, 42) very like the one we envision for oocytes (23). Oocytes do not have the capability of catalyzing nonhomologous end joining, which is often the predominant process in mammalian cells (29). Even when long homologies are not available, the secondary recombinants that arise are generated by the overlap of shorter, imperfect homologies very near molecular ends (11). *Xenopus* eggs and egg extracts support end joining (3, 27, 32), but their capacity for homologous recombination has not been critically tested. The absence of end joining in oocytes has made it easier to study homologous recombination without interference from homology-independent processes.

The DSBR model was originated to explain observations in *S. cerevisiae*, and it provides a good description of what happens in transformation with linear DNAs that have homology to chromosomal sites (38). Perhaps surprisingly, some types of DSB-stimulated events in yeast cells appear to be nonconservative. When a break is introduced by the HO nuclease into one copy of directly repeated, mutant *lacZ* genes, either in a chromosome (30) or on an extrachromosomal plasmid (31), deletions make up 85% of the recovered products in mitotic cells. In a *rad52* mutant, an HO cut in tandemly repeated sequences is relatively efficiently repaired with concomitant deletion formation (26). It has been shown that 3′ tails are produced at HO cuts (43) and during normal meiotic recombination (5, 16, 37), providing evidence for a 5′→3′ exonuclease in yeast cells like that in oocytes. It seems probable that these 3′ tails could engage either in invasion events, as required by the DSBR model, or in annealing events, if complementary single strands are exposed near the molecular ends.

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