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Recombination between a resident plasmid and the chromosome following irradiation of the radioresistant bacterium *Deinococcus radiodurans*

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Abstract

Interplasmidic and intrachromosomal recombination in *Deinococcus radiodurans* has been studied recently and has been found to occur at high frequency following exposure to ionizing radiation. In the current work, we document plasmid-chromosome recombination following exposure of *D. radiodurans* to 1.75 Mrad (17.5 kGy) ^{60}Co, when the plasmid is present in the cell at the time of irradiation. Recombination is assayed using both physical and allelic polymorphisms of homologous genes in the plasmid and chromosome. Recombination was found to be largely, but not entirely, *recA*-dependent. Crossovers occur frequently, and a significant fraction of these are non-reciprocal.

Keywords: DNA repair; Reciprocal recombination; Nonreciprocal recombination; γ-Irradiation; *recA*; Single-strand annealing

1. Introduction

Deinococcus (formerly *Micrococcus*) *radiodurans* (*D.r.*) and its close relatives share extreme resistance to the lethal and mutagenic effects of ionizing and UV radiation and many chemical agents that damage DNA. The dramatic DNA damage resistance of *D.r.* has been shown to be due to exceedingly efficient DNA repair; however, the mechanisms involved are understood poorly (for reviews, see Moseley, 1983; Minton, 1994). RecA-dependent recombinational repair appears to be central to survival (Gutman et al., 1994; Daly and Minton, 1995), with *D.r.* containing the multiple chromosomes necessary to provide redundant information for recombinational repair: four chromosomes in stationary phase, and as many as 10 during exponential phase (Hanson, 1978).

We have recently proposed a mechanism to explain how *D.r.* efficiently brings together homologous fragments to recombine with each other during repair following high dose irradiation (Minton and Daly, 1995). In this model, recombinational repair occurs in the context of chromosomes that are always aligned during normal growth. The resulting chromosomal proximity ensures immediate access to substrate during recombinational repair. We have also suggested that such a chromosomal complex should be accessible to other extrachromosomal fragments liberated by DSBs, and that such mobile fragments may be responsible for patching gaps in a partially restored chromosomal complex (Daly and Minton, 1996).

To address the question of whether DNA that is not associated with a chromosome is accessible to the chromosome during repair, we investigated recombination between homologous chromosomal and plasmidic fragments following high dose irradiation. By using physical and genetic polymorphisms present in the homologous regions, we have been able to monitor: (i) crossovers by Southern blotting; and (ii) genetic recombination as manifested by the recovery of drug resistance in *D.r.*

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Abbreviations: Ap, ampicillin; bp, base pair(s); cfu, colony-forming unit(s); Cm, chloramphenicol; *D.r.*, *Deinococcus radiodurans*; DSB, DNA double-strand break; *E.c.*, *Escherichia coli*; kb, kilobase(s) or 1000 bp; Km, kanamycin; Mb, megabases(s) or 1 000 000 bp; nt, nucleotide(s); *recA*, gene encoding RecA protein; ^{R}, resistance/resistant; ^{S}, sensitive/sensitivity; SSA, single-strand annealing; Tc, tetracycline; TGY, 0.8% tryptone/0.1% glucose/0.4% yeast extract (medium); wt, wild type.

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2. Results and discussion

2.1. pMD300 and the chromosomal insertion in MD367 (recA$^+$) and MD360 (recA)

pMD300 is an *Escherichia coli* (*E.c.*)-*D.r.* shuttle vector that provides the substrate to assess plasmid-chromosome recombination in *D.r.* when it is resident in either MD367 (MD367/pMD300) or MD360 (MD360/pMD300). The chromosomal and plasmid constructions differ in the segments derived from *Escherichia coli* (*E.c.*) vectors, as follows: (i) drug resistance: pMD300 contains and expresses the *cat* gene (Cm^R), while MD367 and MD360 do not; (ii) physical polymorphisms: pMD300 and the chromosomes are physically polymorphic in the region of the *tet* gene, containing flanking segments that differ upstream of *tet* (a 2.2-kb insertion of rat cDNA in pMD300) and downstream of *tet* (a 695-bp *Pvu*II-*Sty*I deletion of the pBR322-derived portion of the chromosomes MD367 and MD360 (Fig. 1A)). These polymorphisms allow for assessment of recombination occurring between the plasmid and the chromosome by restriction cleavage and Southern blot analysis (Fig. 1B); and (iii) allelic polymorphisms in the *tet* gene (heteroalleles): The chromosomes of the wt and *recA* derivatives are Tc^S as a result of the presence of the *Xho*I-5 mutation in the *tet* gene at nt position 1268 of pBR322, while pMD300 is Tc^S as a result of the *Xho*I-2 mutation at pBR322 nt position 339 in the *tet* gene (Fig. 1B). These mutations are 929 bp apart, allowing for detection of recombination in this region by selecting for Tc^R.

2.2. Physical polymorphisms following irradiation

Cells were grown to stationary phase and irradiated anaerobically to 1.75 Mrad. Under these conditions approx. 40% of the *recA*$^+$ cells survive as determined by cfu, while all *recA* cells are killed (extrapolates to 10^{-15} survival). This exposure produces about 150 DSBs per chromosome (Daly et al., 1994a). Because of the extreme resistance of *D.r.* to irradiation, doses compatible with a high degree of cellular survival inflict severe plasmid damage. In MD367/pMD300 (*recA*$^+$), pMD300 is repaired and can transform *E.c.* efficiently. However, there was no recovery of pMD300 transforming activity in MD360/pMD300 (*recA*) despite the availability of chromosomal homologous sequences (not shown).

Southern blot analysis of genomic DNA using a *Pvu*II digestion and a radiolabeled *tet* gene probe permitted the assessment of plasmid-chromosome crossovers within the specific window labeled 'X' in Fig. 1B. At various times following irradiation, genomic DNA was purified, cleaved with *Pvu*II, and analyzed by Southern blot using the radiolabeled *tet* gene as probe (Fig. 2). The relative amounts of the parental bands indicative of the plasmid (4.6 kb) and the chromosome (5.2 kb) that have not undergone recombination within this window vary with time following irradiation (Fig. 2). Densitometric analysis (not shown) of the 5.2 kb chromosomally derived *PvuII* hybridizing fragments indicated that there was an 8-fold reduction in chromosomal DNA content per cell, following irradiation. This reduction in hybridization occurring between the time of irradiation and subsequent recovery is consistent with our hypothesis that a single chromosome is regenerated from the original 4–10 identical chromosomes present per cell in *D.r.* It can also be seen from Fig. 2 that while the intensity of the hybridizing 5.2-kb fragment (chromosomal) varies considerably, the plasmid-derived 4.6-kb band does not. This can be explained by the fact that every chromosome will have been broken by DSBs following irradiation due to its size (3.1 Mb); all chromosome fragments, therefore, will be very vulnerable to exonucleolytic attack before repair. The plasmids are very small in comparison (30 kb) and, while they will sustain abundant DNA damage, they are less likely to have DSBs and, thus, less likely to be subject to degradation.

The recombinant bands of 7.3 and 2.35 kb, arising from exchanges between chromosome and plasmid fragments, are clearly evident in MD367/pMD300, but only faintly visible in MD360/pMD300 (Fig. 2). Densitometric analysis of the 46 h *recA*$^+$ (MD367/pMD300) time point of the autoradiogram shown in Fig. 2 (*Pvu*II digestion) gives abundant signal at 7.3 and 2.35 kb (Fig. 3). However, in the *recA* strain MD360/pMD300 only little plasmid-chromosome recombination occurred and the recombinant bands of 2.35 and 7.3 kb are barely detectable (Fig. 3).

A single reciprocal crossover generates one 2.35-kb and one 7.3-kb fragment, since both double helices become reconnected (albeit to the other duplex). A non-reciprocal recombination event generates either one 2.35-kb or one 7.3-kb fragment, since only one helix is crossed, leaving a DSB behind. A striking finding in Fig. 3 is that the molar ratio of the 7.3-kb band is barely half that of the 2.35-kb band, indicating the possible occurrence of numerous non-reciprocal crossovers. In this regard the plasmid-chromosome recombination system might be mimicking chromosome-chromosome recombination in which non-reciprocal recombination occurs frequently (Daly and Minton, 1995, 1996).

We have reported recently that *D.r.* wt and *recA* strains have the capacity to mend as many as one third of their DSBs by a RecA-independent single-strand annealing (SSA) pathway (Daly and Minton, 1996) that occurs when single-stranded homologous fragments from separate chromosomes hybridize. Immediately after irradiation this organism renders substantial regions of its DNA single-stranded, thereby initiating

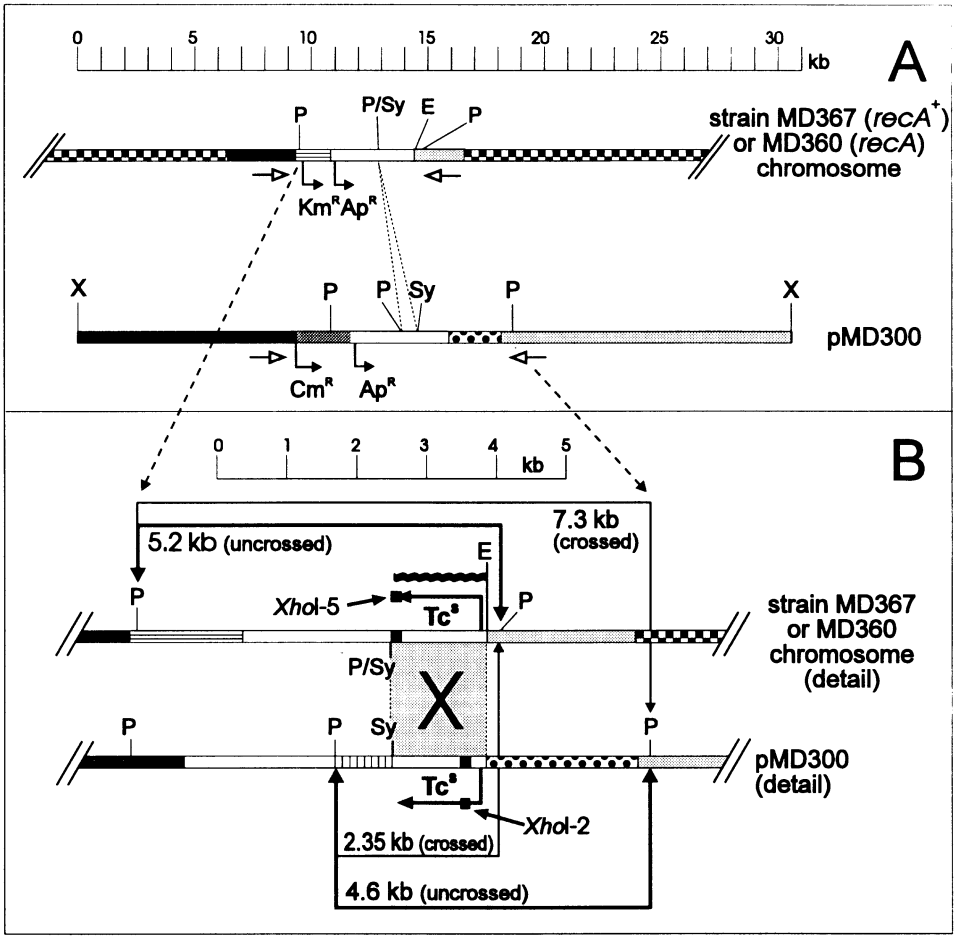

Fig. 1. Plasmid and chromosomal map, functions, probes, and restriction fragments indicative of recombination. The construction of pMD300 (Daly et al., 1994b) and the *D.r.* strains MD367 and MD360 (Daly and Minton, 1995) have been described previously. MD367 and MD360 are the same except that the former is *recA*$^+$, while the latter is *recA.* The *D.r.* strain used in this investigation is strain R1, which is most commonly studied. The strain constructions and the plasmid construction used segments of *D.r.* strain SARK plasmid DNA from pUE10 and pUE11 (Daly et al., 1994a), plasmids that lack homology to each other and the *D.r.* strain R1 chromosome. They are not naturally present in strain R1. (**A**) Full-length map of plasmid pMD300 and detail of *D.r.* R1 derivatives MD367 or MD360 chromosome showing the relevant region for recombination studies. In *D.r.*, pMD300 replicates autonomously because of the plasmid origin of replication derived from the *D.r.* strain SARK plasmid pUE10 (light gray segment) (Daly et al., 1994a). pMD300 contains a selectable Cm^R marker in *D.r.* due to the presence of promoter sequences derived from another SARK plasmid, pUE11 (blackened segment) adjacent to a *cat* (Cm^R) (dark gray segment). pMD300 can also express *tet* (if the *tet* gene is wt) due to the pUE10-derived promoter that is upstream of the *tet* gene. The *tet* gene is in a DNA sequence derived from pBR322 (white segment), which includes the *bla* (Ap^R) gene and the pBR322 origin of replication. Thus, pMD300 and its Tc^R derivatives can replicate autonomously in *Ec.* (Ap^R or Tc^R selection), as well as in *D.r.* (Cm^R or Tc^R selection). The open-headed arrows indicate the direction and approximate location of the pUE10 and pUE11 promoters. The polka-dotted (not checkered) segment in pMD300 is a rat cDNA fragment from pGABI (Daly et al., 1994b) that contains a portion of a rat biliary glycoprotein cDNA. This segment creates a physical polymorphism lacking any homology to the chromosomal construction, and which can be used to trace recombinational events. The deinococcal chromosomal flanking DNA, that is interrupted by the plasmid sequences in MD367 (*recA*$^+$) and MD360 (*recA*), is represented by a checkered pattern. The chromosome in *D.r.* strain R1 expresses Km^R, because of a *D.r.* promotor derived from the *D.r.* SARK plasmid pUE11 (blackened segment) that is joined, in the chromosome to *E.c. aphA* gene (horizontally hatched segment). The chromosomal segment can express Tc^R (if the *tet* gene is wt) because of the promoter from SARK plasmid pUE10 (light gray segment). A physical polymorphism relative to pMD300 is present in the chromosomal insertion in the form of a 695-bp *Pvu*II-*Sty*I deletion created in the portion of the construction derived from pBR322 (white segment). The 695-bp fragment that is deleted from the pBR322 portion of the chromosomal construction is indicated by dotted lines connecting the *Pvu*II-*Sty*I region in pMD300 to the *Pvu*II/*Sty*I fusion site in the chromosome. This deletion does not affect function, but, like the rat DNA in pMD300, provides a traceable physical polymorphism. Restriction sites: E, *Eco*RI; P, *Pvu*II; P/Sy, *Pvu*II-*Sty*I fusion; X, *Xba*I. (**B**) Detail of MD367/MD360 insertions and pMD300, showing expected *Pvu*II restriction digestion fragments made visible by use of the *tet* gene probe. The *Pvu*II restriction digestion gives fragments indicative of crossover (or lack of crossover) within the region of homology (gray field labeled 'X'). The probe, indicated by a wavy black line, is the 1.27-kb *Eco*RI-*Xho*I fragment from pRDK39 (Doherty et al., 1983; Daly et al., 1994b; Daly and Minton, 1995) that contains the pBR322 *tet* gene. Parental fragments, visible by autoradiography, are 5.2 kb (from the chromosome) and 4.6 kb (from pMD300). Fragments indicative of recombination made visible by autoradiography are 7.3- and 2.35 kb. The *Xho*I-2 mutation consists of an 8 bp *Xho*I linker inserted in a *Taq*I site at nt position 339 in the *tet* gene of pBR322, and renders the gene Tc^S (Doherty et al., 1983; Daly et al., 1994b). The *Xho*I-5 mutation consists of the same linker inserted at a *Taq*I site at nt position 1268 of the *tet* gene of pBR322 and also inactivates the gene (Tc^S) (Doherty et al., 1983; Daly et al., 1994b). Restriction enzyme abbreviations and segments as in panel A.

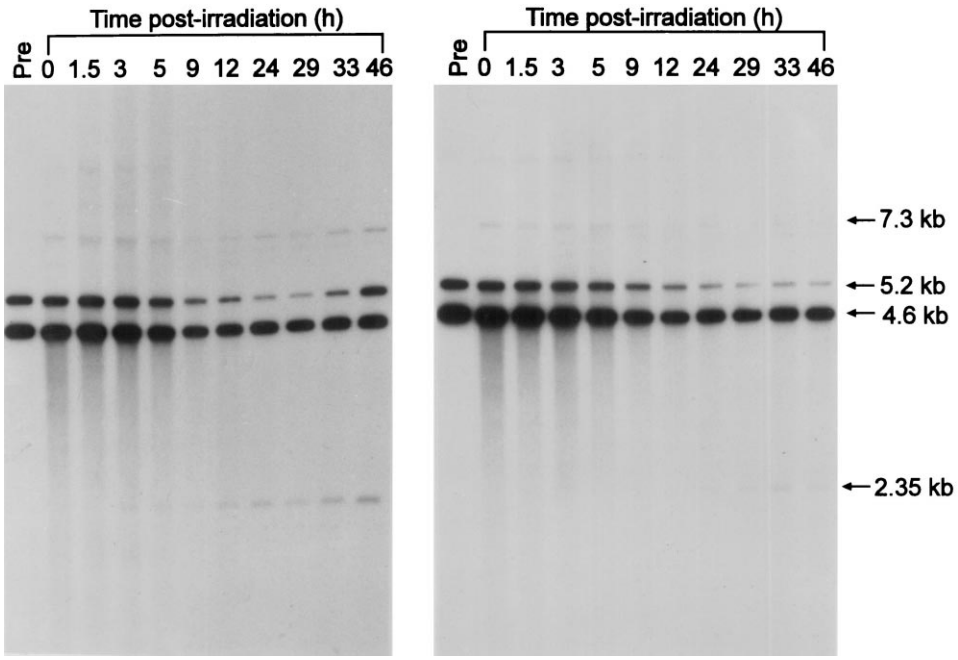

Fig. 2. Southern blot showing production of recombinant bands following irradiation of MD367/pMD300 (*recA*$^+$) (left) and MD360/pMD300 (*recA*) (right). Cells were grown overnight at 32°C to early plateau phase cells in TGY in the presence of 3 μg Cm/ml. The cells were then irradiated without change of broth on ice with ^{60}Co to 1.75 Mrad (1.75 kGy) at a dose rate of 1 Mrad/h, diluted 1:50 in fresh TGY medium and incubated at 32°C with aeration in the absence of antibiotics. DNA was prepared from samples at the indicated times by the miniprep technique and cleaved with *Pvu*II. Each lane contains the DNA from 3×10^6 cells (about 200 ng per lane in unirradiated cells) as determined by hemocytometer count. Electrophoresis was in a 0.7% agarose gel for 18 h at 55 V prior to blotting and probing of the blot with the 1.27-kb fragment of the *tet* gene purified from pRDK39, that had been radiolabeled with [α-^{32}P]dCTP by the random priming method (Daly et al., 1994b; Daly and Minton, 1995).

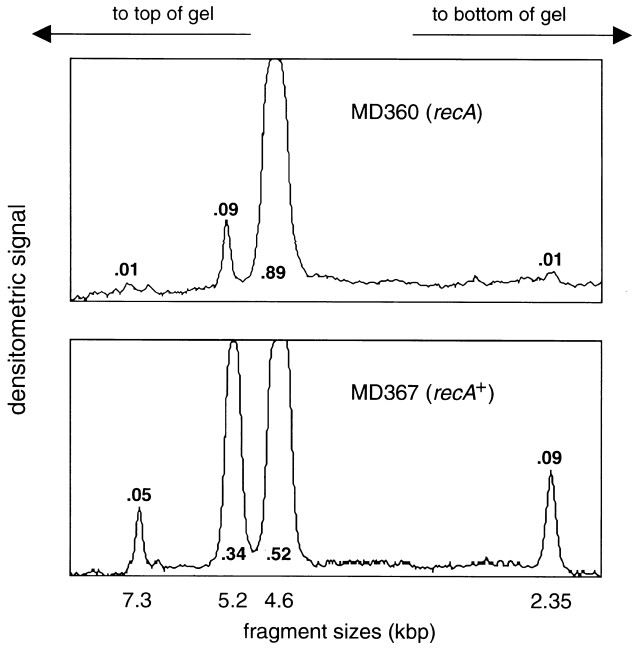

Fig. 3. Densitometric film scanning of 46 h gel lanes shown in Fig. 3 by use of the gel scan module NIH 1.5.4. using reflectance densitometry with a Hewlett Packard Scan Jet 4C. The two parental bands are located centrally, bracketed by the two outer recombinant bands, which are at the top and bottom of each lane. The fragment length of the parental and recombinant bands are shown at the bottom. The amount of signal in each band, relative to the total signal, is indicated above or inside the peaks.

the annealing process. If SSA is also occurring in MD360/pMD300 and MD367/pMD300, then restriction endonuclease sites (such as *Pvu*II) that lie in regions of single-stranded DNA will not be cut, resulting in larger bands. Such bands are visible during the first 5 h of recovery (Fig. 2). For example, if a region of pBR322 (white segment of Fig. 1) is rendered single-stranded at the *Pvu*II site, it will not be cleaved there by *Pvu*II. However, the flanking *Pvu*II sites (if intact) will then give a 7.5-kb fragment which is indistinguishable from the recombinant 7.3-kb fragment. There is also a larger fragment present in both wt and *recA* (visible for the first 5 h) that can be explained in a similar way. The existence of low abundance recombinant bands seen in MD360/pMD300 (*recA*) might also be due to *recA*-independent SSA reactions that can result in structures identical to a *recA*-dependent crossover (Haber, 1992; Daly and Minton, 1996).

2.3. *Recombination determined by* tet *heteroalleles*

MD367/pMD300 (*recA*$^+$) cells that had recovered 46 h post-irradiation were plated on Tc-selective TGY agar. 2.2% of this population was Tc^R. The presence of plasmid-borne Tc^R was investigated by transforming 1 μg of total *D.r.* DNA taken from 46 h post-irradiation cells into *E.c.* DH10B using Ap^R selection. These cells were then replica plated for Tc resistance. It was found

that 3 out of a total of 587 Ap^R colonies were Tc^R. Thus, no more than 0.5% (3/587) of *D.r.* cells contained a Tc^R plasmid. Hence, 4-fold more cells (2.2%) were Tc-resistant due to chromosomally encoded Tc^R as compared to plasmid-encoded Tc resistance (0.5%). One possibility to account for this difference is that there is a greater length of homology downstream of the *tet* gene (including pBR322 and deinococcal sequences) as compared to upstream, which could bias against the upstream location for the second crossover (which is necessary for generating a Tc^R plasmid) (Fig. 1A). Alternatively, this bias may be another manifestation of non-reciprocity in recombination in *D.r.*

2.4. Conclusions

(1) Plasmid-chromosome recombination following a high-dose of ionizing irradiation of *D.r.* is largely RecA-dependent.
(2) Homologous plasmid and chromosomal fragments generated by irradiation are accessible to each other in wt *D.r.* and can recombine during DNA repair. This suggests that *D.r.* treats plasmid and chromosome fragments the same way and supports our previous assertion that interplasmidic recombination (Daly et al., 1994b) is an accurate model system for chromosomal repair (Daly and Minton, 1995).
(3) Some plasmid-chromosome recombination appears to be non-reciprocal. Non-reciprocal recombination has been reported in *D.r.* during recombinational repair of its chromosome (Daly and Minton, 1995). This non-reciprocal recombination may be occurring by a *recA*-independent SSA pathway that begins immediately after DNA damage in *D.r.*, as previously proposed (Daly and Minton, 1996).

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