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Roles of the recG gene product of Escherichia coli in recombination repair: Effects of the ΔrecG mutation on cell division and chromosome partition.

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Running title: recG gene function in recombination repair

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Abstract

The products of the *recG* and *ruvAB* genes of *Escherichia coli* are both thought to promote branch migration of Holliday recombination intermediates by their junction specific helicase activities in homologous recombination and recombination repair. To investigate the *in vivo* role of the *recG* gene, we examined the effects of a *recG* null mutation on cell division and chromosome partition. After UV irradiation at a low dose (5J/m²), Δ*recG* mutant formed filamentous cells with unpartitioned chromosomes. A mutation in the *sfiA* gene, which encodes the SOS-inducible inhibitor of septum formation, largely suppressed filamentation of *recG* mutant cells, but did not prevent the formation of anucleate cells. The sensitivity to UV light and the cytological phenotypes after UV irradiation of a *recA recG* double mutant were similar to a *recA* single mutant, consistent with the role of *recG* which is assigned at a later stage in recombination repair than that of *recA*. The *recG ruvAB* and *recG ruvC* double mutants were more sensitive to UV and showed more extreme phenotypes concerning filamentation and chromosome nondisjunction, both after UV irradiation and during exponential growth than either *recG* or *ruv* single mutant. The *recG polA12* (Ts) mutant, which is temperature sensitive in growth, formed filamentous cells with centrally located chromosome aggregates when grown at nonpermissive temperature. These results support that RecG plays a role in processing recombination intermediates in recombination repair.
Introduction

*recG* mutants are slightly more deficient in homologous recombination and slightly more sensitive to UV light than wild type strain (Storm et al., 1971; Lloyd and Buckman, 1991). These findings suggest that the *recG* gene product is involved in recombination and recombination repair. Furthermore, *recG* mutants with additional mutations in one of the *ruv* (*ruvA*, *ruvB* and *ruvC*) genes are more deficient in both conjugational and transductional recombination and more sensitive to UV light than either *recG* or *ruv* single mutants (Lloyd, 1991). These findings suggest that the function of the *recG* gene product overlaps those of the *ruv* gene products in recombination and recombination repair.

SOS-inducible RuvA and RuvB proteins form a functional complex which promotes ATP-dependent migration of Holliday junctions by catalyzing strand exchange reaction and enlarges the heteroduplex region in homologous recombination *in vitro* (Shiba et al., 1991; Iwasaki et al., 1992; Parsons et al., 1992; Tsaneva et al., 1992). The product of the *ruvC* gene, which is not regulated by SOS system (Sharples et al., 1990; Sharples and Lloyd, 1991; Takahagi et al., 1991), resolves Holliday junctions to give mature recombinant molecules by introducing nicks at or near the crossover junction in the strands with the same polarity (Dunderdale et al., 1991; Iwasaki et al., 1991; Sharples and Lloyd, 1991).

The role of RecG in recombination is less well understood. The *recG* gene belongs to the *spoT* operon (Lloyd and Sharples, 1991). RecG protein also binds specifically to Holliday junctions, possesses a DNA-dependent ATPase activity, and dissociates synthetic Holliday junctions in an ATP-dependent manner (Lloyd and Sharples, 1993; Whitby et al., 1994). Like RuvAB, RecG also drives branch migration of Holliday junctions made by RecA (Sharples et al., 1994). The results suggest that RecG plays a role in branch migration of Holliday junctions, like RuvAB complex, in homologous recombination and recombination repair. However, RecG differs from RuvAB in the polarity of DNA unwinding (3' to 5' with respect to single-stranded DNA) and in promoting branch migration in a direction opposite from that promoted by RecA.
in the presence of RecA. When RecA-free recombination intermediates are used as the substrates, RecG promotes branch migration in both directions (Whitby et al., 1993; Whitby and Lloyd, 1995). Based on the above finding and the fact that RecG has no junction-specific endonuclease like RuvC, it has been proposed that RecG resolve the junctions by a reverse branch migration mechanism (Whitby et al., 1993).

Compared to biochemical studies of RecG protein, studies on the role of recG in vivo are rather scarce. To elucidate the function of recG in vivo, we constructed, in this work, a recG null mutation in a way not to alter the expression of the neighboring genes and cytologically studied the effects of the recG mutation on cell division and chromosome partition.
Materials and Methods

General Methods. Standard methods for *E. coli* genetics are as described by Miller (1992). Recombinant DNA techniques are as described by Sambrook et al. (1989).

Strains, media and growth conditions. The *E. coli* strains used in this study are listed in Table 1. Using the 7.6-kb *KpnI* DNA fragment derived from the Kohara’s λ #571 clone containing the *recG* gene (Lloyd and Sharples, 1991), a Δ*recG* allele was constructed on plasmid (Fig. 1). To delete the *recG* gene, a 1.9-kb *PstI*-*BglII* fragment which contains most of the *recG* coding region was replaced with a synthetic DNA fragment which contains the sequences ligatable to the ends generated by digestion with *PstI* and *BglII* at the ends, the authentic ATG translation initiation codon of the *recG* gene and the identical nucleotide sequence of the *recG* upstream region (Fig. 1a). Subsequently, a *km* gene cassette, which is derived from pUC4K as the 1.4-kb *PstI* fragment, was inserted into the *BglII* site, after the *BglII* and *PstI* digested DNA ends were converted to blunt ends by treatment with mungbean nuclease. A DNA fragment containing this *recG* deletion allele (*recG100::km*) was introduced by transformation with linear DNA into D30I strain (Russell et al., 1989), giving HRS1997 strain. The isogenic derivatives of particular strains with the *recG* (Table 1) mutations were constructed by P1 phage mediated transduction (Miller, 1992).

Microscopy and photography. To observe cell shapes and nucleoids simultaneously, cells were stained with 4', 6-diamidino-2-phenyl-indole (DAPI) solution, observed with a Nikon OPTIPHOT-2 microscope with fluorescence and phase contrast capabilities, and photographed using Fuji color reversal film PROVIA 400.
Results

Defects of recG mutant cells in chromosome partition after UV irradiation.

Extensive filamentation (Otusji et al., 1974) and chromosome nondisjunction of UV irradiated ruv mutants have been observed (K. I., H. I. and H. S., unpublished data). We studied the change in morphology of the recG100::km mutant after UV irradiation (Fig. 2). After a low dose of UV irradiation (5J / m²), both the wild type and recG mutant cells formed very long filaments during a 2 hr incubation. The wild type cells contained well-segregated chromosomes distributed evenly throughout the filaments, whereas the recG mutant produced both filamentous cells with centrally located chromosome aggregates and anucleate cells. By 4 hr after UV irradiation, wild type cells resumed cell division and returned. On the other hand, the recG mutant gave rise to heterogeneous cell population; filamentous cells with aggregated chromosomes (~30%), normal-sized anucleate cells (~10%) and normal-sized cells containing chromosome (~60%). These results suggest that efficient processing of the recombination repair intermediates is required for proper chromosome partition. recG mutant was much less sensitive to UV than are the ruv mutants (Fig. 3; Lloyd, 1991). We compared the morphology between the recG and ruv mutant cells grown for 4 hr after UV irradiation at the doses which gave 10% survivals for these strains. The recG and ruv strains produced anucleate cells 10% and 80%, respectively (data not shown). The increase in the UV dosage did not increased the anucleate cell formation in the recG strain.

sfiA dose not prevent production of anucleate cells. sfiA is a SOS inducible gene and the product is a cell division inhibitor which antagonizes the function of a septum forming protein, FtsZ (Huisman et al., 1984). The recG and ruv mutants express higher basal levels of a SOS gene than the wild type strains (Lloyd and Buckman, 1991; Asai and Kogoma, 1994). We examined the effects of sfiA mutation on the morphology of the UV irradiated recG mutant cells (Fig. 2) By 2 hr after UV irradiation (5J / m²), sfiA mutant cells became slightly elongated, but they were shorter than the wild type cells and their chromosomes were well partitioned. sfiA mutation ameliorated elongation of
the wild type and recG mutant cells, but it did not prevent the formation of chromosome aggregation or production of anucleate cells in the recG mutant.

ΔrecA prevents chromosome nondisjunction and anucleate cell formation in recG cells. If the formation of filamentous cells with centrally located chromosome aggregates and subsequent production of anucleate cells in the recG mutant cells were due to the defect in processing of recombination intermediates, recA mutations should suppress the phenotypes of the recG mutant by preventing the formation of recombination intermediates. The UV sensitivity of recA recG double mutant was indistinguishable from that of recA single mutant (Fig. 3). The UV-irradiated recA recG double mutant cells exhibited very similar morphological phenotypes as those of the UV irradiated recA single mutant cells (Fig. 2). UV irradiation promoted filamentation and formation of anucleate cells in both recA single and recA recG double mutants. However chromosomes were well partitioned in the elongated cells in these recA mutants in contrast to the recG single mutant.

Morphology of exponentially growing recG mutant cells. We studied the morphology of exponentially growing recG mutant cells. About 40% of the recG mutant cells were longer than 4 µm, whereas only 0.5% of the wild type cells were longer than 4 µm (Fig. 4). Since basal the level of the SOS regulated sfiA gene expression was two- to four-fold higher in a recG mutant than in a wild type strain (Lloyd and Buckman, 1991; Asai and Kogoma, 1994), the filamentation in the recG mutant is due most likely to the sfiA-dependent cell division inhibition. However, the recA or the sfiA mutation only partially suppressed the filamentation of recG mutant cells (Fig. 4). These results suggest the existence of a sfiA- and recA-independent cell division inhibition mechanism.

We determined the proportion of anucleate cells in the recG mutant and its derivatives (Fig. 5). The recG mutant produced more anucleate cells (1.5%) than the wild type strain (0.15%) but less than did the recA strain (7%). The levels of anucleate cell production in the recA mutant and the recA recG mutant (8%) were very similar.
These results are consistent with the current notion that RecA initiates homologous recombination and RecG process recombination intermediates (See reviews; Whitby et al., 1995; Shinagawa and Iwasaki, 1996).

**Synergistic effects of recG and ruv mutations.** recG ruv double mutants were more sensitive to DNA damaging agents and much more defective in the recombination than either recG and ruv single mutants (Lloyd, 1991). The recG ruvC double mutant was highly sensitive to UV and almost as sensitive as the recA and recA recG strains (Fig. 3). A large proportion of the exponentially growing recG ruv mutants formed very long cells with centrally aggregated chromosomes and produced many anucleate cells (4.5%) (Figs. 4, 5, 6). UV irradiation increased markedly filamentous cells and anucleate cells, which constituted great majority of the cell population (Fig. 6). These phenotypes of the recG ruv mutants were more similar to the ruv mutants than to the recG mutant, although the double mutants showed slightly more severe morphological phenotypes than ruv mutants (K. I., H. I. and H. S., unpublished data). These results are consistent with the current notion that the recG and ruv genes function in the alternative pathways for processing the recombination intermediates and suggest that the defects in the processing interfere the partition of chromosomes. The results also suggest that homologous recombination is taking place rather frequently in the exponentially growing cells in the absence of DNA damaging agents.

**Morphology of recG polA12 (Ts) mutant cells grown at nonpermissive temperature.**

The polA gene encodes DNA polymerase I which possesses 5' to 3' polymerase, 5' to 3' exonuclease and 3' to 5' exonuclease activities. DNA polymerase I encoded by the polA12 mutant allele is active at 30 °C in the nick translation activity (5' to 3' polymerase and 3' to 5' exonuclease) but defective at 42 °C (Monk and Kinross, 1972). The polA12 strains with additional mutations in recA, recB, recC or ruv are temperature sensitive for growth (Monk and Kinross, 1972; K. I., H. I. and H. S., unpublished data). recG polA12 double mutants are also temperature sensitive (Hong et al., 1995). We studied the
morphology of recG polA12 cells grown at nonpermissive temperature (42 °C)(Fig. 7). By 2 hr after the temperature shift, recG polA12 double mutant cells became highly filamentous and the chromosomes were aggregated in the central regions of the cells as in the UV irradiated recG strain. The polA12 single mutant grown at 42 °C was normal in size and chromosome partition (Fig. 7). Further incubation of recG polA12 cells at nonpermissive temperature resulting in increase of anucleate filamentous cells (10%) but not of small anucleate cells as did the ruv polA12 mutants (K. I., H. I. and H. S., unpublished data). Introduction of sfiA mutation did not rescue the temperature-sensitivity of recG polA12 mutant cells (data not shown), indicating that the temperature-sensitivity of the double mutant is not due to the inhibition of cell division by induced SfiA protein.
Discussion

Cell division inhibition in the recG mutant and SOS response. We showed that exponentially growing recG mutant cells produced more filamentous cells than the wild type cells and that both on sfiA and a recA mutation partially suppressed the filamentation (Fig. 2, 4). SOS induced SfiA protein inhibits the septation by blocking the formation of the FtsZ ring (Bi and Lutkenhaus, 1993; Lutkenhaus and Mukherjee, 1996). It has been reported that the SOS response is chronically induced in a recG mutant (Lloyd and Buckman, 1991; Asai and Kogoma, 1994). Therefore, the spontaneous filamentation of recG mutant cells is most likely caused by the SOS induced SfiA-dependent cell division inhibition. When the recG mutant was irradiated with a low dose of UV (5 J/m²), both the proportion of anucleate cells and the cell size were increased substantially. Although cell division inhibition of UV-irradiated recG strain is due in large part to the SOS-induced SfiA protein, sfiA, sfiA recG, recA and recA recG strains were still elongated after UV irradiation. The extents of UV-induced cell elongation in these strains were similar. These results suggest that there is sfiA- and recA-independent pathway(s) of cell division inhibition. sfiA and SOS-independent mode of inhibition of cell division has been reported in E. coli inhibited for DNA replication (Jaffé et al., 1986). We do not know the relevance of this observation to the present observation.

RecA protein complexed with single stranded DNA (nucleoprotein filament) is thought to be an active form that promotes autocleavage of the LexA repressor, leading to the induction of a set of SOS genes including sfiA (Higashitani et al., 1992; 1995; Sasanfer and Roberts, 1990; Walker, 1996). Genetic and biochemical studies suggest that RecG protein processes the Holliday recombination intermediates as does the RuvABC. Probably, the recG mutant, like ruv mutants, is prone to accumulate single stranded DNA and branched DNA molecules due to the defect in processing recombination intermediates (Nakayama et al., 1994). These DNA molecules could be targeted for by RecA protein, resulting in the generation of the SOS signal.
Alternatively, R-loops expected to accumulate in the recG mutant might be responsible for the induction of the SOS response. RecA may bind to the displaced single-stranded DNA in the R-loop. Consistent with this idea is an rnhA mutant, lacking the RNase HI activity and thus defective in resolving R-loops, chronically induces the SOS response and RecG protein can also resolve the R-loops (Kogoma et al., 1993; Vincent et al., 1996; Fukuoh et al., 1997).

**Inhibition of chromosome partition and production of anucleate cells in the recG mutants.** UV-irradiated recG mutant cells were morphologically similar to the recG polA12 mutant cells grown at nonpermissive temperature, which contained nonpartitioned chromosomes aggregated in the center of the highly elongated cells (Figs. 2, 7). However, the recG mutant produced much less anucleate cells than the ruv mutants (Fig. 5; K. I., H. I. and H. S., unpublished data).

According to the current models of homologous recombination and recombination repair, RecBCD protein complex enters double-strand break DNA ends and generates recombinogenic DNA molecules with single stranded ends by its helicase and nuclease activities. RecA protein binds to the single stranded region to form nucleoprotein filament, promotes homologous pairing and initiates strand exchange reaction, leading to the formation of D-loops and probably Holliday recombination intermediates (See review; Kowalczykowski et al., 1994). These recombination intermediates are processed via RuvABC and RecG pathways and converted to mature recombinant molecules (See reviews; Whitby et al., 1995b; Shinagawa and Iwasaki, 1996; West, 1996). That the recG mutant and ruv mutants showed similar phenotypes are consistent with the above model. However, recG mutant produced much less anucleate cells than ruv mutants, which might reflect to the minor role of RecG in the processing of recombination intermediates. The marked defects in chromosome partition observed in the recG ruv mutants indicate that recombination (and probably double strand breaks) is rather frequently occurring during normal growth, which has gone undetected with the wild type strain.
References


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strains containing genetically linked alternating antibiotic resistance elements for

recombination in *Escherichia coli*. IV. Isolation and characterization of


Table 1. Used *E. coli* strains in this study

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<tr>
<th>Strain</th>
<th>Relevant Genotype</th>
<th>Reference or Source</th>
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<td>AB1157</td>
<td><em>rec</em>, <em>ruv</em>, <em>sfiA</em></td>
<td>Bachmann (1972)</td>
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<td>CAG18495</td>
<td><em>zih</em>-35::<em>Tn10</em></td>
<td>Singer et al. (1989)</td>
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<td>D301</td>
<td><em>recD1903</em>:<em>Tc</em></td>
<td>Russell et al. (1989)</td>
</tr>
<tr>
<td>DM2569</td>
<td>Δ(<em>srl-recA</em>)306::<em>Tn10</em></td>
<td>Ennis et al. (1985)</td>
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<td>George et al. (1975)</td>
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<td>As AB1157 but Δ<em>ruvC100</em>:<em>cat</em></td>
<td>Saito et al. (1995)</td>
</tr>
<tr>
<td>HRS1997</td>
<td>As D301 but Δ<em>recG100</em>:<em>km</em></td>
<td>This study</td>
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<td>As AB1157 but Δ<em>recG100</em>:<em>km</em></td>
<td>AB1157 X P1 HRS1997</td>
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<tr>
<td>HRS2006</td>
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<td>AB1157 X P1 DM2569</td>
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<td>HRS2102</td>
<td>As D301 but Δ<em>ruvAB100</em>:<em>cat</em></td>
<td>K. Ishioka unpublished</td>
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<td>HRS2302</td>
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<td>HRS7060</td>
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<td>MM386 X P1 CAG18495; <em>Tc</em>&lt;sup&gt;f&lt;/sup&gt;, <em>UV</em>&lt;sup&gt;s&lt;/sup&gt;</td>
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<td>MM386</td>
<td><em>polA12</em></td>
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**Figure 1** Construction of recG100::km allele. A 7.6 kb DNA fragment, which contains the recG gene, was subcloned from the Kohara’s λ 571 clone into plasmid pACH191. a, The 1.9 kb PstI-BgIII fragment which contains most of the recG coding region was replaced with a synthetic DNA fragment (boxed sequence). b, A km gene cassette is inserted into the BglII site. See text for the details. Abbreviations: B, BglII; K, KpnI; P, PstI.

**Figure 2** Morphological changes after UV irradiation of recG derivatives. Exponentially growing cells at 37 °C in LB medium were irradiated with UV at time 0. Samples were taken at the times indicated above the figure, fixed stained DAPI, and photographed. Scale bar indicates 10 µm. The strains are indicated in the left of the figure. wild type (A to C), AB1157; recG (D to F), HRS2000; sfiA (G to I) GC579; sfiA recG (J to L), HRS2406; recA (M to O), HRS2006; recA recG (P to R), HRS4900. wild type, sfiA, recG and sfiA recG strains were irradiated UV with 5 J / m² and recA, recA recG strains done with 0.5 J / m².

**Figure 3** UV sensitivity of strains carrying combination of mutations in rec and ruv genes. The strains used were: AB1157 (wild type), open circle; HRS2000 (recG), closed circle; HRS2302 (ruvAB), open square; HRS1100 (ruvC), closed square; HRS2006 (recA), open triangle; HRS4900 (recA recG), closed triangle; HRS2505 (recG ruvC), diamond. The data shown are based on four independent experiments.

**Figure 4** Distribution of cell length in the recG derivatives. Cells growing exponentially in LB at 37 °C were fixed, stained and photographed. The length of the cells in the photographs were measured. The back, densely dotted, sparsely dotted, and blank areas represent the cells with lengths >10 µm, 10-5 µm, 5-4 µm and <4 µm, respectively. wild type, AB1157; recG, HRS200; sfiA, GC579; sfiA recG, HRS2406;
recA, HRS2006; recA recG, HRS4900; recG ruvAB, HRS2502; recG ruvC, HRS2502; ruvAB, HRS2302; ruvC, HRS1100.

**Figure 5** Proportion of anucleate cell in the recG derivatives.
Samples were prepared and photographed as described in Fig. 2, and the proportion of anucleate cells was counted in the photograph. The same set of strains as in Fig. 4 was used.

**Figure 6** Morphological changes after UV irradiation of the recG ruvAB and recG ruvC mutants. The figure shows the recG ruvAB HRS2502 (A to C) and recG ruvC HRS2505 (D to F). Experimental procedures were as described in the legend to Fig. 2, except that these were irradiated by UV at 0.5 J / m² which gave 10% surviving fraction. The sampling times were indicated above the figure and the strains were indicated in the left of the figure.

**Figure 7** Morphological changes of recG polA12 (Ts) grown at nonpermissive temperature. polA12 (Ts); HRS7064 (A to C) and recG polA12 (Ts); HRS3100 (D to F) strains were grown in LB at 30 °C to OD₉₀₀=0.1 and they were shifted to 42 °C. Samples were taken at the times indicated and photographed as in Fig. 2.
Fig. 1 Ishioka et al.
Wild type:

- A: 0 h
- B: 2 h
- C: 4 h

RecG:

- D: 0 h
- E: 2 h
- F: 4 h

SfiA:

- G: 0 h
- H: 2 h
- I: 4 h

SfiA recG:

- J: 0 h
- K: 2 h
- L: 4 h

RecA:

- M: 0 h
- N: 2 h
- O: 4 h

RecA recG:

- P: 0 h
- Q: 2 h
- R: 4 h

Fig. 2 Ishioka et al.
Fig. 3 Ishioka et al.
Fig. 4 Ishioka et al.
Fig. 5 Ishioka et al.
RecG ruvAB

RecG ruvC

Fig. 6 Ishioka et al.
Fig. 7 Ishioka et al.

polA12 42 °C

recG polA12 42 °C