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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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 / m^2), *ΔrecG* 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 *Kpn*I DNA fragment derived from the Kohara's λ #571 clone containing the *recG* gene (Lloyd and Sharples, 1991), a *ΔrecG* allele was constructed on plasmid (Fig. 1). To delete the *recG* gene, a 1.9-kb *Pst*I-*Bgl*II fragment which contains most of the *recG* coding region was replaced with a synthetic DNA fragment which contains the sequences ligatable to the ends generataed by digestion with *Pst*I and *Bgl*II 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 *Pst*I fragment, was inserted into the *Bgl*II site, after the *Bgl*II and *Pst*I 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 D301 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 / m^2), 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 / m^2), *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.

Δ*recA* 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 formaiton 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 / m^2), 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 Hollidy 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.

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

Strain	Relevant Genotype	Reference or Source
AB1157	*rec*$^+$, *ruv*$^+$, *sfiA*$^+$	Bachmann (1972)
CAG18495	*zih-35*::*Tn10*	Singer et al. (1989)
D301	*recD1903*::*Tc*	Russell et al. (1989)
DM2569	*Δ(srl-recA)306*::*Tn10*	Ennis et al. (1985)
GC579	*rec*$^+$, *ruv*$^+$, *sfiA11*	George et al. (1975)
HRS1100	As AB1157 but *ΔruvC100*::*cat*	Saito et al. (1995)
HRS1997	As D301 but *ΔrecG100*::*km*	This study
HRS2000	As AB1157 but *ΔrecG100*::*km*	AB1157 X P1 HRS1997
HRS2006	As AB1157 but *Δ(srl-recA)306*::*Tn10*	AB1157 X P1 DM2569
HRS2102	As D301 but *ΔruvAB100*::*cat*	K. Ishioka unpublished
HRS2302	As AB1157 but *ΔruvAB100*::*cat*	AB1157 X P1 HRS2102
HRS2406	As GC579 but *ΔrecG100*::*km*	GC579 X P1 HRS2000
HRS2502	As HRS2000 but *ΔruvAB100*::*cat*	HRS2000 X P1 HRS2102
HRS2505	As HRS2000 but *ΔruvC100*::*cat*	HRS2000 X P1 HRS1100
HRS3100	As HRS7064 but *ΔrecG100*::*km*	HRS7064 X P1 HRS1997
HRS4900	As HRS2000 but *Δ(srl-recA)306*::*Tn10*	HRS2000 X P1 DM2569
HRS7060	As MM386 but *zih-35*::*Tn10*	MM386 X P1 CAG18495; Tc^r, UV^s
HRS7064	AS AB1157 *polA12 zih-35*::*Tn10*	AB1157 X P1 HRS7060; Tc^r, UV^s
MM386	*polA12*	Monk and Kinross (1972)

Figure legends

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 *Pst*I-*Bgl*II 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 *Bgl*II site. See text for the details. Abbreviations: B, *Bgl*II; K, *Kpn*I; P, *Pst*I.

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 / m^2 and *recA*, *recA recG* strains done with 0.5 J / m^2.

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 / m^2 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 OD_{600}=0.1 and they were shifted to 42 °C. Samples were taken at the times indicated and photographed as in Fig. 2.

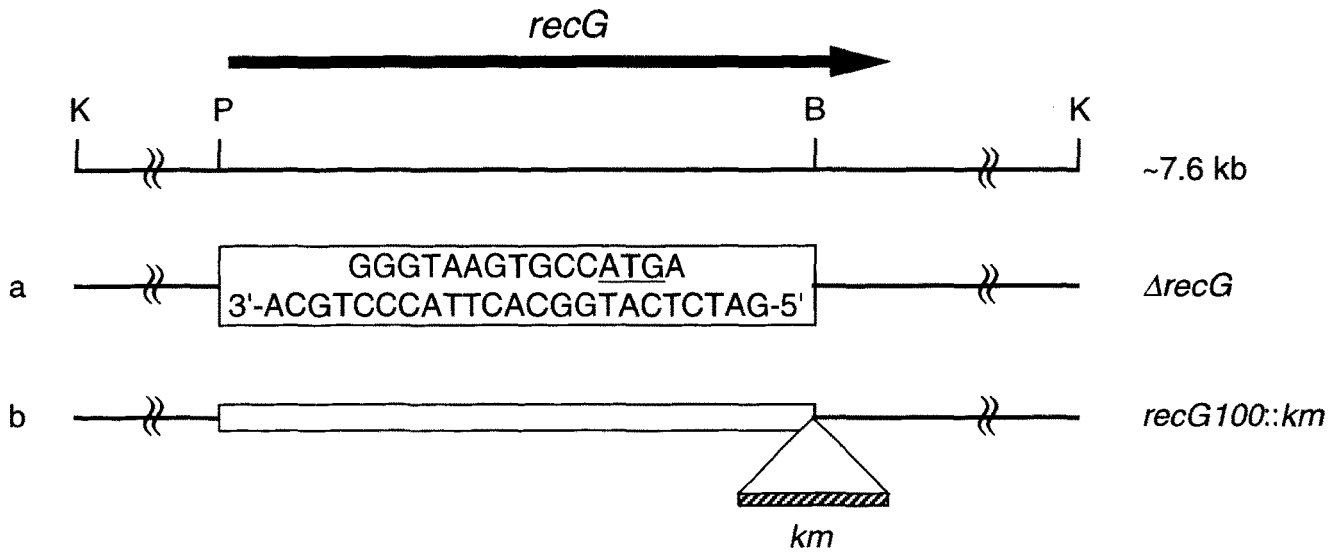

Fig.1 Ishioka et al.

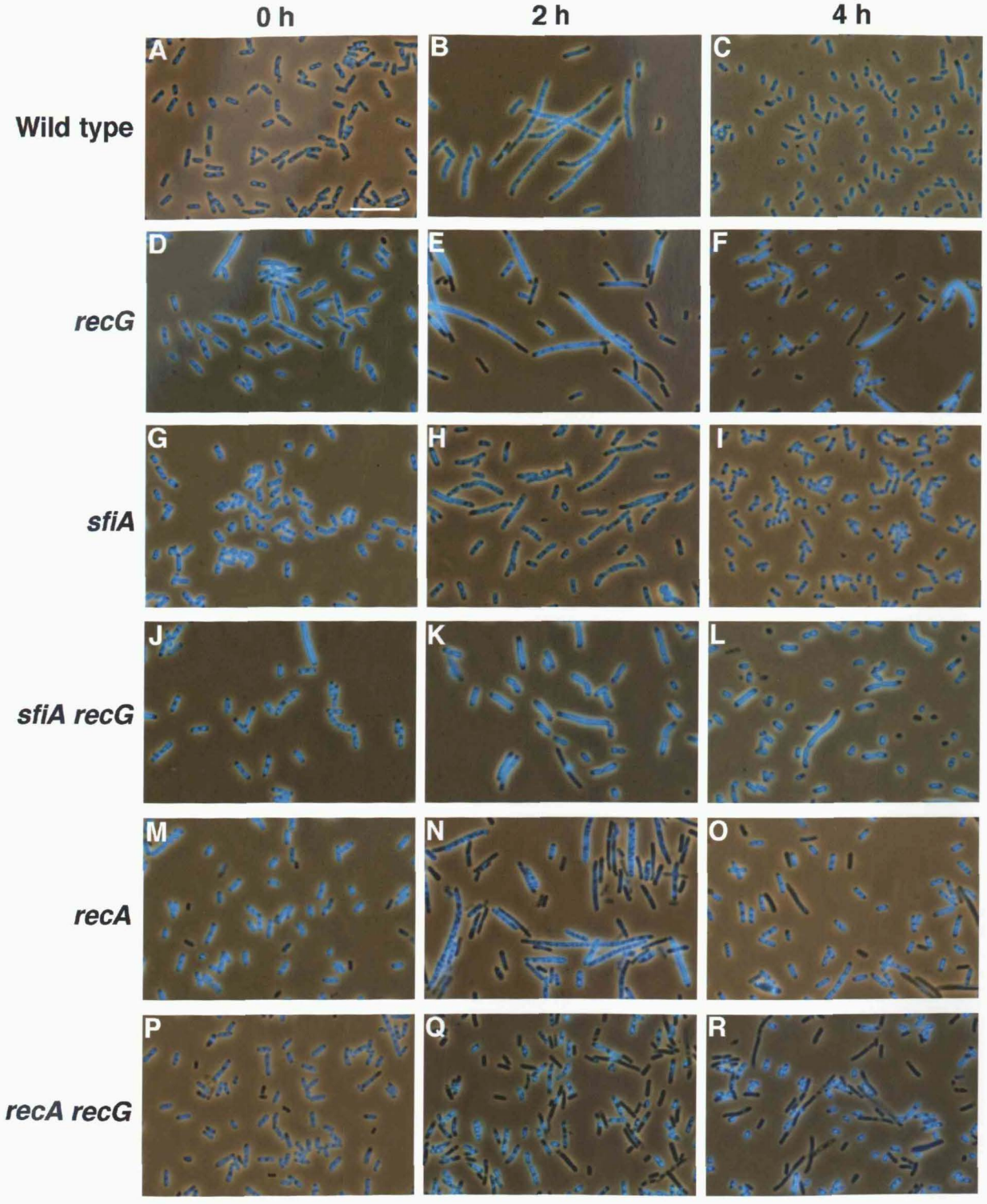

Fig.2 Ishioka et al.

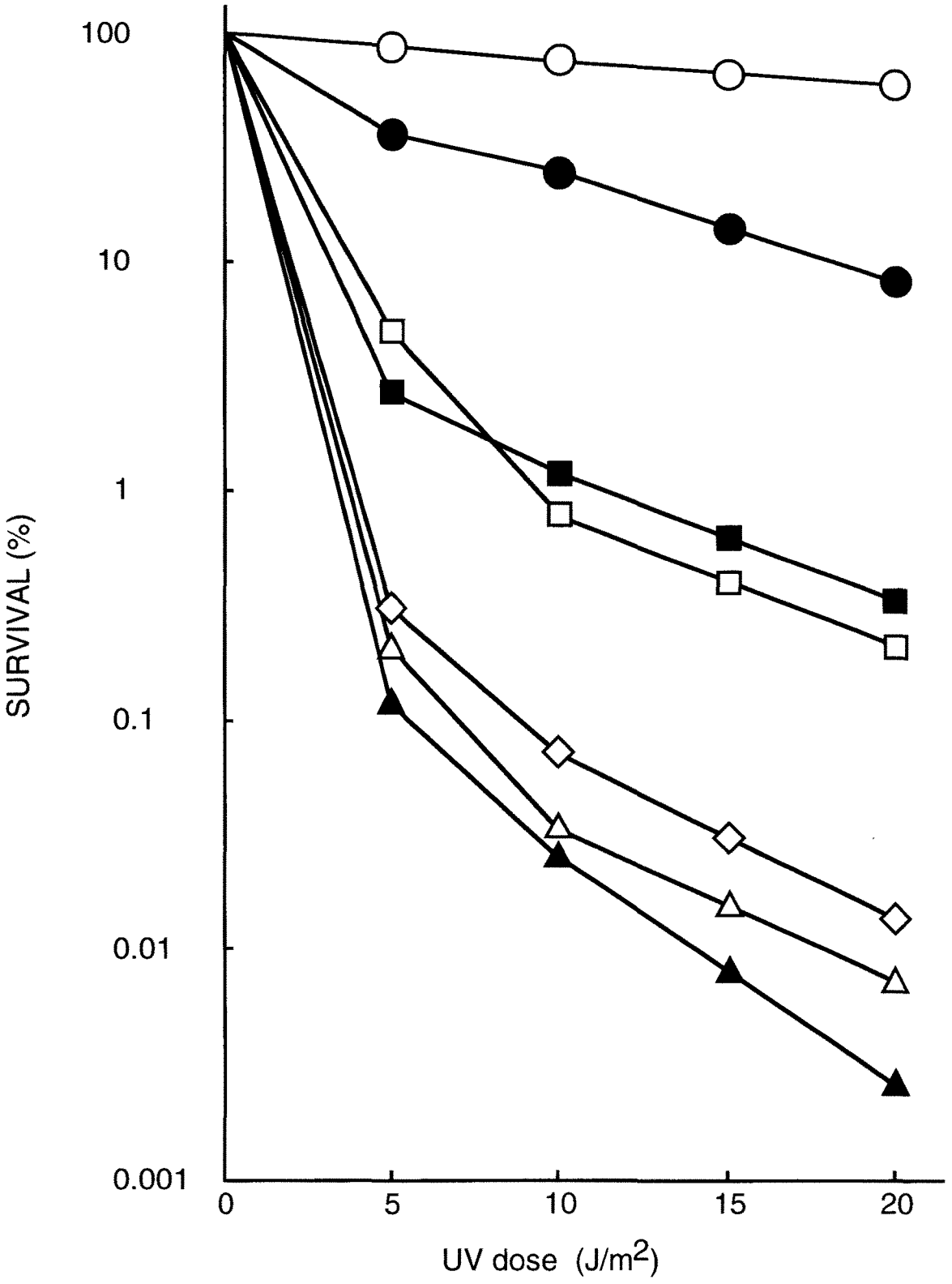

Fig.3 Ishioka et al.

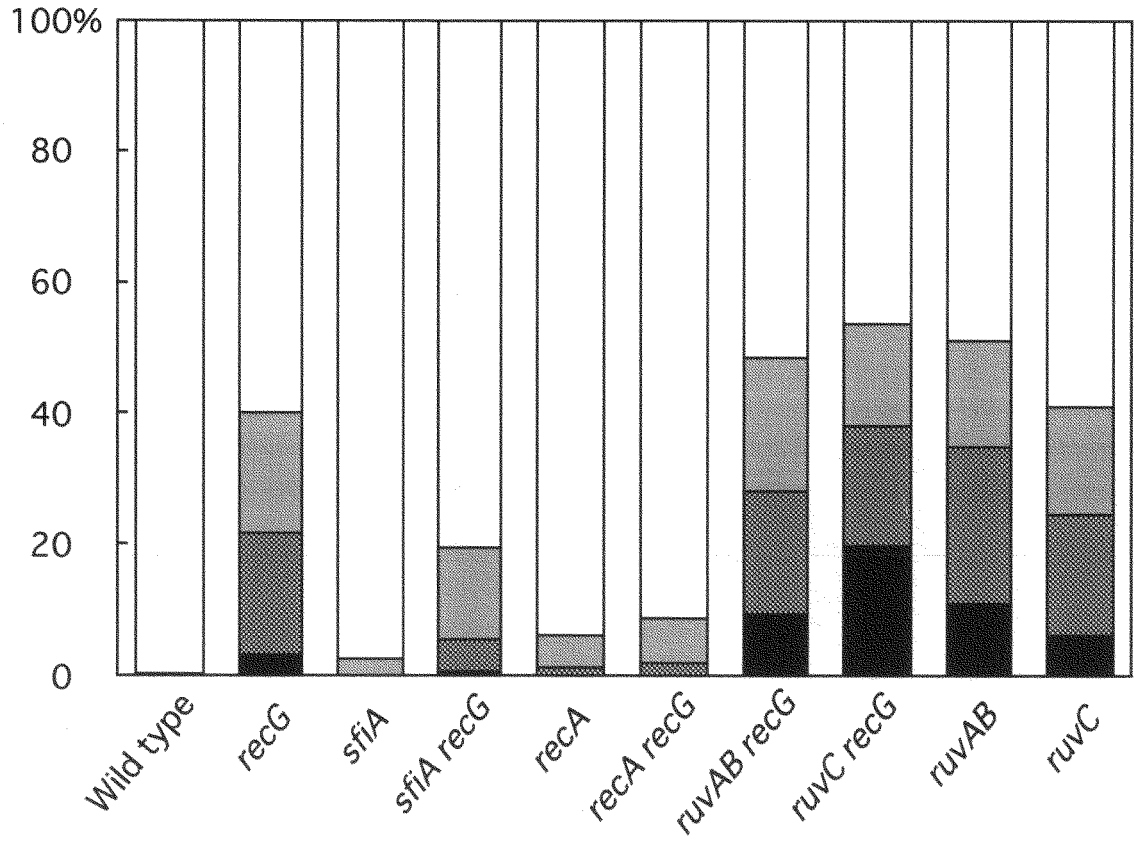

Fig.4 Ishioka et al.

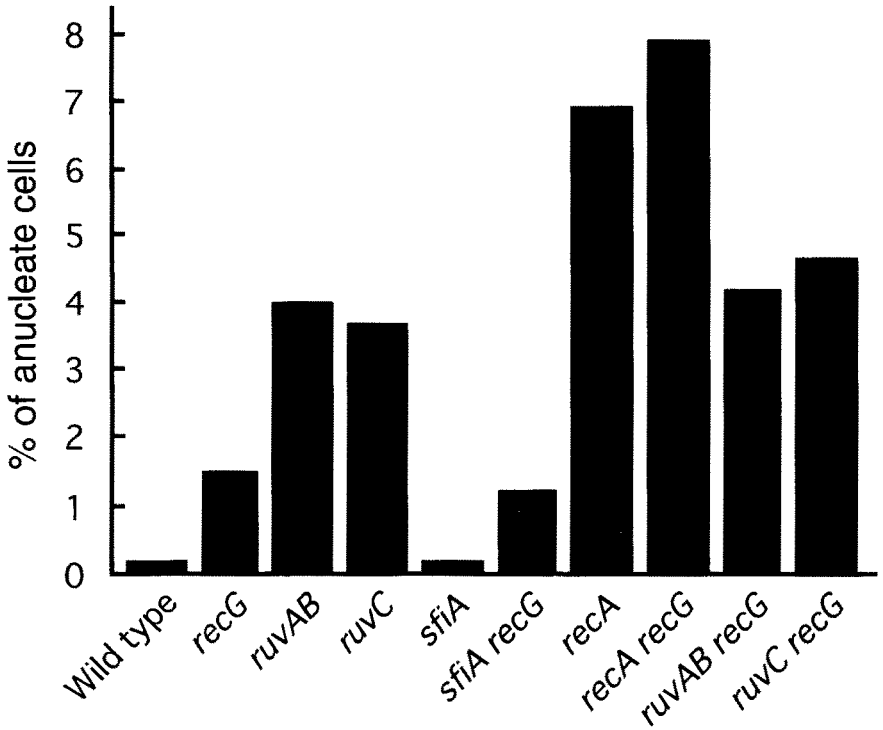

Fig.5 Ishioka et al.

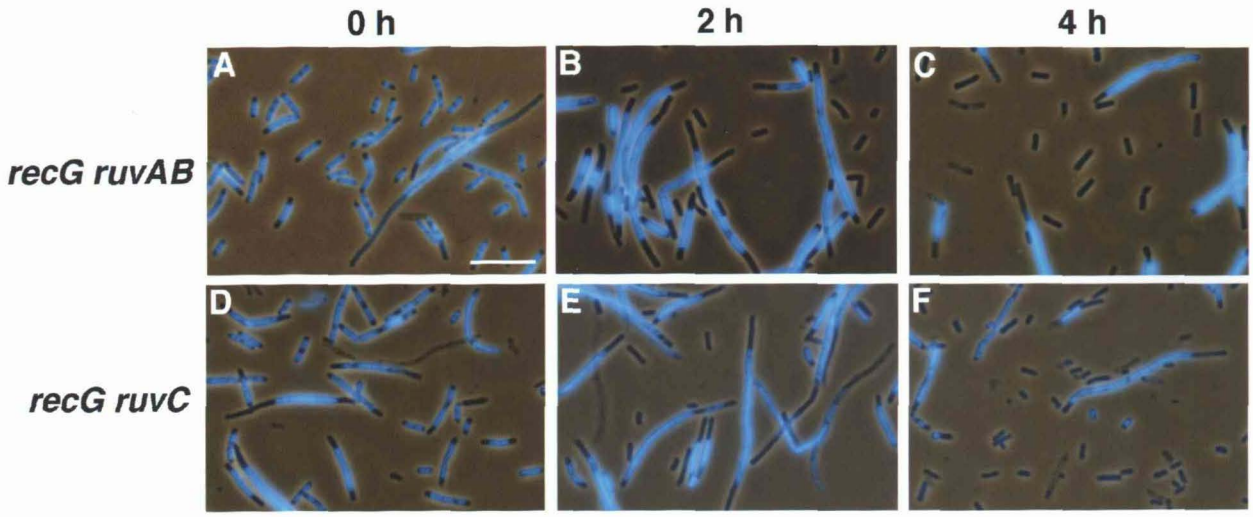

Fig.6 Ishioka et al.

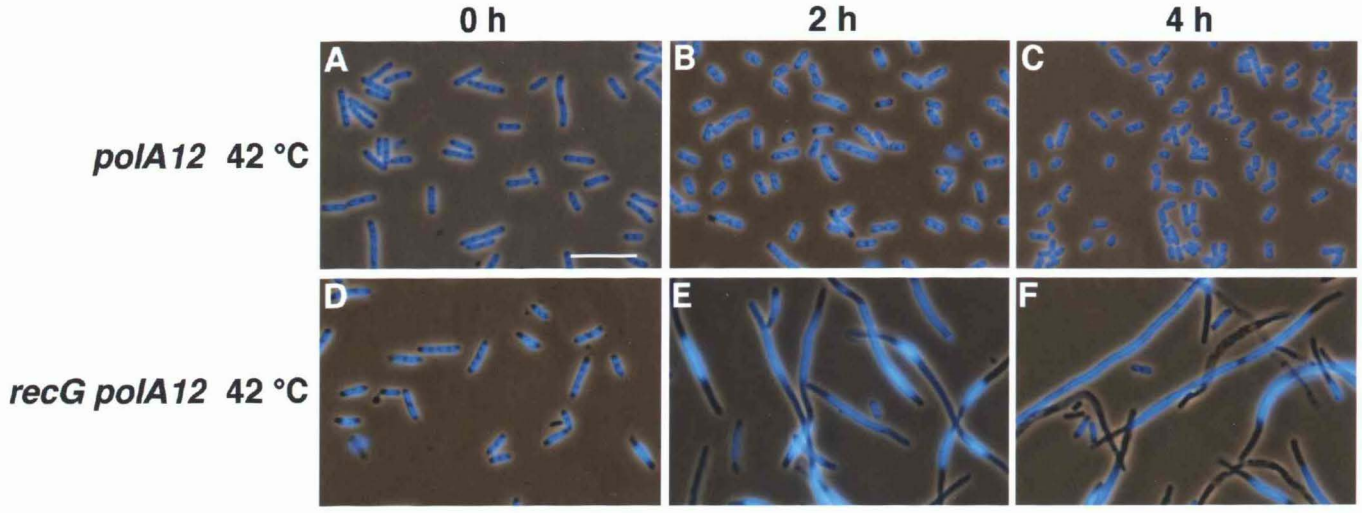

Fig.7 Ishioka et al.