Recombination and UV resistance of *Escherichia coli* with the cloned *recA* and *recBCD* genes of *Serratia marcescens* and *Proteus mirabilis*: evidence for an advantage of intraspecies combination of *P. mirabilis* RecA protein and RecBCD enzyme

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In *Escherichia coli*, constituents of the main recombination pathway are provided by the genes *recA* (RecA protein) and *recBCD* (RecBCD enzyme). Recombination in conjugation experiments and repair of UV damage of *E. coli* mutants deleted for *recA*, for *recBCD* or for *recA* plus *recBCD* were restored, although to different degrees, by the cloned *recA* and *recBCD* genes from *Serratia marcescens* or *Proteus mirabilis*. When both recombination enzymes were from the same species, repair and recombination efficiencies had the order *E. coli* > *S. marcescens* > *P. mirabilis*. However, the *P. mirabilis* *recA* plus *recBCD* genes resulted in higher levels of repair and recombination than those obtained with one component from *P. mirabilis* (*recA* or *recBCD*) and the other from *E. coli* or *S. marcescens*. The data provide evidence for the similarity of RecABCD pathways of recombination among enteric bacteria and suggest an *in vivo* advantage of an intraspecies combination of *P. mirabilis* RecA protein and RecBCD enzyme over interspecies combinations. This could point to a cooperation between these basic recombination enzymes. The molecular processes which could be involved are discussed.

Introduction

The major recombination pathway in *Escherichia coli* following DNA transfer by conjugation or transduction is the RecBCD pathway (Clark, 1973). Genetic studies have revealed the dependence of this pathway on RecA protein and RecBCD enzyme, but also on other activities of DNA metabolism including DNA gyrase, DNA ligase, single-stranded DNA binding protein (SSB) and DNA polymerase I (for reviews, see Mahajan, 1988; Smith, 1988). Mutations in *recA* reduce recombination proficiency by about four orders of magnitude, mutations in *recB* or *recC* by about two (Willets & Clark, 1969). *In vitro* RecA protein catalyses several reactions including homologous alignment of single- or double-stranded DNA with another homologous double-stranded DNA molecule and the ATP-dependent single-strand transfer between these substrates (for reviews, see Cox & Lehman, 1987; Radding, 1988). In addition, RecA protein accelerates renaturation of complementary single strands. RecBCD enzyme, also termed exonuclease V, was found to have ATP-dependent single-

and double-strand exonuclease and single-strand endonuclease activities (for a review, see Telander-Muskavitch & Linn, 1981). Moreover, the RecBCD enzyme is an ATP-dependent helicase that during unwinding of DNA recognizes and cleaves specific octanucleotide sequences called Chi (Ponticelli *et al.*, 1985; Schultz & Smith, 1986). The latter two activities have been correlated to the enzyme's function in recombination (see Smith, 1988). Chi sites have been shown to enhance RecBCD-dependent recombination in their vicinity (Smith & Stahl, 1985). As has been confirmed by *in vitro* experiments (Roman & Kowalczykowski, 1989*b*; Wang & Smith, 1989), RecA protein can use the single-stranded DNA produced by RecBCD enzyme helicase activity to promote the strand transfer reaction.

In the well-established genetic system of *E. coli* homologous recombination can be measured by parasexual processes such as conjugation and transduction or by phage crosses. In most other bacterial species similar processes have not been elaborated or even been detected so far. Therefore, a measurement of recombination proficiency in these organisms is impossible.

Although the existence of RecA proteins and of RecBCD enzymes in various bacterial species (Telander-

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Muskavitch & Linn, 1981; Smith, 1988; McKittrick & Smith, 1989) predicts a broad distribution of the RecBCD pathway of recombination among prokaryotes, the existence of such pathways in bacteria other than *E. coli* has not yet been shown. We have investigated recombination by RecA plus RecBCD enzymes of the two enterobacteria *Serratia marcescens* and *Proteus mirabilis*. To circumvent the problem of not having a means to measure recombination in these species at present, we transformed the cloned *recA* and *recBCD* genes of *S. marcescens* and *P. mirabilis* into *E. coli* deleted for these genes. In the transformants we determined recombination proficiency provided by the heterologous enzymes using the conjugation system of *E. coli*. In addition, the UV sensitivity of the transformants was used as a measure of their DNA repair potential dependent on recombination functions.

The experimental approach of reconstituting the recombination system of one species in the cells of another species allows us to study a new aspect. This is whether the enzymes performing the different steps of recombination work with the same efficiency if they come from different species as if they come from the same species. The data obtained provide evidence for a cooperation of RecA protein with RecBCD enzyme in the sense that with both enzymes from the same species (*P. mirabilis*) higher efficiencies of repair and recombination were obtained than with interspecies combinations including only one *P. mirabilis* enzyme.

Methods

Bacterial strains and plasmids. Table 1 contains the description of bacterial strains and plasmids used in this study. Strains were constructed by P1 transduction (Miller, 1972). In the main studies mutants deleted for *recA* and/or *recBCD* (strains JV1, JV3, JV10 and JV12) were used. They were derived from strain WA675 (Weichenhan & Wackernagel, 1988) and therefore are isogenic, apart from the noted genotype differences (Table 1).

DNA manipulations. Plasmid DNA was isolated by alkaline lysis (Birnboim & Doly, 1979). Restriction enzymes were purchased from Boehringer Mannheim, Pharmacia and Gibco/BRL and were used as recommended by the supplier. T4 DNA ligase was isolated as described by Murray *et al.* (1979). Ligations were performed according to Rusche & Howard-Flanders (1985). Standard molecular cloning procedures were used (Maniatis *et al.* 1982).

Media and growth conditions. Strains were grown in TBY broth (1%: 10 g tryptone, 5 g yeast extract, 5 g NaCl) at 37 °C. If needed, thymine was added to a final concentration of 50 µg ml⁻¹. For strains carrying plasmids, the appropriate antibiotics were added to give the following final concentrations: ampicillin (Ap), 40 µg ml⁻¹; chloramphenicol (Cm), 20 µg ml⁻¹; kanamycin (Km), 15 µg ml⁻¹; tetracycline (Tc), 7 µg ml⁻¹. Agar plates containing mitomycin C at a concentration of 1 µg ml⁻¹ were used for rapid discrimination between *recA* and *recA*⁺ as well as between *recBCD* and *recBCD*⁺ cells by replica plating of colonies from transformation plates.

Exponential-phase cultures for conjugation experiments or for determination of UV sensitivity were grown without antibiotics. Since we observed that the formation of exconjugant colonies by antibiotic-resistant cells was somewhat hampered on plates containing antibiotics, the plates for determination of recombinants and exconjugants, or for determination of survivors were prepared without the antibiotics selecting for the presence of *recA* or *recBCD* plasmids. Under these conditions we found that two plasmids (pDV31 and pPG35) were lost in up to 50% of the exconjugants or UV survivors. Therefore, the actual fraction of plasmid-carrying, recombination-proficient cells was determined in each experiment by replica-plating the colonies appearing on plates with non-irradiated cells (UV experiment) or with exconjugants onto media selective for the presence of pDV31 or pPG35. The colony titres of UV survivors and exconjugants were corrected accordingly.

Determination of UV resistance and recombination proficiency. Determination of UV resistance was performed as described by Thoms & Wackernagel (1982). Bacterial matings were performed using the HfrH strain JC158(pML2) (Lovett & Clark, 1983) as donor, which allows the determination of recombination and conjugation frequency in the same cross. The transfer origin is located at 98 min on the *E. coli* chromosome and DNA is transferred in a clockwise direction towards *proA*⁺ (6 min). Conjugation proficiency was determined by the recombination-independent transfer of the mobilizable plasmid pML2, which confers kanamycin resistance to the recipients. For a given recipient strain, the transfer of pML2 was not measurably affected by the presence of one of the plasmids carrying the various *rec* genes. Matings were performed at 37 °C in TBY with a ratio of recipient to Hfr cells of 10. They were interrupted by vigorous vortexing after 40 min to block transfer of *recA*⁺ (59 min) and *recB*⁺*C*⁺*D*⁺ genes (61 min) to the recipient cells (map positions according to Bachmann, 1990). Counterselection against the Hfr strain was done by not providing serine and thiamin in the M9 minimal medium plates for which the Hfr strain is auxotrophic. In each cross 100–200 recombinant colonies (on M9 with thymine and arginine) and exconjugant colonies (on M9 with thymine, arginine, proline and kanamycin) were scored. The recombination frequency of a given strain was calculated by dividing the titre of *pro*⁺ recombinants by the titre of Km^R exconjugants for each recipient strain tested and normalized to the value obtained with *E. coli recA*⁺ *recB*⁺*C*⁺*D*⁺. The standard deviation for these recombination frequencies was calculated from three or more independent experiments.

Results

Cloning of the recA genes of Serratia marcescens and Proteus mirabilis

A genomic library was constructed from *Sau*3AI-digested chromosomal DNA of *S. marcescens* SR41 (Takagi & Kisumi, 1985) by ligation of 23–35 kb fragments into the *Bam*HI site of the mini-F-derived single-copy cosmid pRE432 (Table 1, Fig. 1). To screen for the *recA* gene of *S. marcescens* (*recA*_{Sm}) *E. coli recA* (strain DH5, Table 1) was transformed with these cosmids and replica-plated onto TBY plates with mitomycin C (1 µg ml⁻¹). From one cosmid, which restored mitomycin resistance, *recA*_{Sm} was recloned on a 1.7 kb *Eco*RI fragment into pRE432. The gene was identified by the restoration of UV resistance and

Table 1. Bacterial strains and plasmids

Strain or plasmid	Description	Construction, source or reference
<i>Proteus mirabilis</i> PG1300	<i>arg thr end 1-199</i>	Eitner <i>et al.</i> (1981)
<i>Serratia marcescens</i> Sr41	Wild-type	Takagi & Kisumi (1985)
<i>Escherichia coli</i> strains:		
K12s	<i>supD</i>	Bachmann (1972)
DH5	<i>recA1 endA1 gyrA96 thi-1 supE44 hsdR17</i>	Hanahan (1983)
JC10289	<i>E. coli</i> AB1157 Δ (<i>slrR-recA</i>)306::Tn10	Csonka & Clark (1979)
WA675	Δ (<i>argA-thyA</i>)232 <i>lacZ</i> ::Tn10 <i>proA2</i>	Weichenhan & Wackernagel (1988)
JV1	Δ (<i>argA-thyA</i>)232 <i>proA2</i>	K-12s $\rightarrow$ WA675*
JV10	<i>proA2</i>	K-12s $\rightarrow$ JV1*
JV3	Δ (<i>argA-thyA</i>)232 Δ (<i>slrR-recA</i>)306 <i>proA2</i>	JC10289 $\rightarrow$ JV1*†
JV12	Δ (<i>slrR-recA</i>)306 <i>proA2</i>	JC10289 $\rightarrow$ JV10*†
JC158 (pML2)	<i>serA6 thi-1 lacI-22 rel-1</i> (HfrH) with plasmid pML2 (Km ^R)	Lovett & Clark (1983)
Plasmids:		
pRE432	Ap ^R Cm ^R Tc ^R , 13.5 kb (mini-F origin)	R. Eichenlaub University Bielefeld, FRG
pJE256	Cm ^R Tc ^R , 6.5 kb (mini-F origin)	R. Eichenlaub University Bielefeld, FRG
pNE1	(<i>argA⁺ recBCD⁺ thyA⁺</i>) of <i>S. marcescens</i> in vector pRE432, Ap ^R Cm ^R	Rinken <i>et al.</i> (1991)
pPG35	(<i>recBCD⁺ thyA⁺</i>) of <i>P. mirabilis</i> in vector pJRD184, Ap ^R Tc ^R	Weichenhan & Wackernagel (1988)
pSG1	Cosmid from genomic library of <i>S. marcescens</i> Sr41, <i>recA⁺</i> , Ap ^R Cm ^R (vector pRE432)	This work
pDV1	<i>recA⁺</i> of <i>S. marcescens</i> in vector pRE432, Ap ^R Cm ^R	This work
pDV11	<i>recA⁺</i> of <i>S. marcescens</i> in vector pRE432, Ap ^R Tc ^R	This work
ppM12	Plasmid from genomic library of <i>P. mirabilis</i> PG1300, <i>recA⁺</i> , Ap ^R (vector pBR322)	This work
pDV31	<i>recA⁺</i> of <i>P. mirabilis</i> in vector pJE256, Cm ^R	This work
pSS1	Cointegrate of pDV11 and pNE1, Ap ^R Cm ^R Tc ^R	This work
pps2	Cointegrate of pDV31 and pNE1, Ap ^R Cm ^R	This work

* The donor and recipient strain used for strain construction by P1 transduction are indicated.

† Spontaneous Tc-sensitive mutants of the originally Tc-resistant transductants, which have probably lost Tn10, were isolated by the procedure of Maloy & Nunn (1981) with minor modifications.

recombination proficiency of an *E. coli* *recA* deletion mutant (see following sections) and by hybridization with a probe made from a 0.7 kb *EcoRI*-*NcoI* fragment of the *E. coli* *recA* gene (Sancar *et al.*, 1980) (data not shown). While this work was in progress, cloning of the *recA* genes of two other *S. marcescens* strains was published. In strain SM6 the gene was also found on a 1.7 kb *EcoRI* fragment (Ball *et al.*, 1990), while no *EcoRI* restriction sites were found near to the *recA* gene of strain SM250 (Liao & Liu, 1989), indicating heterogeneity between the strains at least in intergenic regions.

Cloning of the *recA* gene of *P. mirabilis* (*recA_{Pm}*) became necessary because the existing clones were lost after the sequencing of the gene (Akaboshi *et al.*, 1989) was completed (Stephen West, personal communication). A genomic library of *P. mirabilis* PG1300 (Eitner *et al.*, 1981) was constructed with chromosomal DNA fragmented with *Sau3AI* to a size of 10–15 kb and cloned into the *Bam*HI site of pBR322 (Bolivar *et al.* 1977). Screening of clones on mitomycin C medium (see previous paragraph) identified one plasmid from which, after a partial *Sau3AI* digest, a 4.2 kb fragment covering *recA* was cloned into the *Bam*HI site of the mini-F-derived single-copy vector pJE256 (Table 1, Fig. 1a). As

for the *recA* gene of *S. marcescens* (previous paragraph), the presence of *recA_{Pm}* on this plasmid was confirmed by the restoration of UV resistance and recombination proficiency of a *recA* deletion mutant of *E. coli* (see following sections) and by hybridization with the *E. coli* *recA* gene probe (data not shown). Furthermore, restriction mapping (Fig. 1a) was consistent with the previously published map (Eitner *et al.*, 1981) and with the data obtained by sequencing (Akaboshi *et al.* 1989).

UV resistance conferred upon *E. coli* by heterologous *recA* and *recBCD* genes

The plasmids with *recA_{Sm}* (pDV11) or *recA_{Pm}* (pDV31) increased the UV resistance of an *E. coli* *recA1* mutant (strain DH5) only to an intermediate level. At 60 J m⁻² the survival of *E. coli* wild-type was about 15%, whereas that of pDV11 or pDV31 transformants of DH5 was about 1% (survival of DH5 without plasmid: < 10⁻⁵). It has been shown that the *recA1* allele is dominant over the wild-type allele, resulting in negative complementation (Yancey & Porter, 1984). Accordingly, when the *recA* gene was deleted from *E. coli* (strain JV12), the foreign *recA* genes restored the repair capacity to much higher

Fig. 1. Construction of single-copy plasmids carrying the *recA* genes of *P. mirabilis* (a) and *S. marcescens* (b) from plasmids of genomic libraries (top row). The vectors (represented by filled sections) are pBR322 in pPM12, pJE256 (single-copy) in pDV31 and pRE432 (single-copy) in pSG1 and pDV11. The open sections represent insert DNA; the subcloned parts are emphasized by a third line. The transcription direction of *recA_{Pm}* was deduced from *EcoRI* and *ClaI* restriction sites according to Akaboshi *et al.* (1989), that of *recA_{Sm}* was deduced from *EcoRI*, *NcoI* and *PstI* restriction sites according to Ball *et al.* (1990). Abbreviations: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; N, *Nco*I; P, *Pst*I; S, *Sau*3AI (only relevant sites indicated); B/S, *Bam*HI-*Sau*3AI ligation site; Ap, Cm, Tc, ampicillin, chloramphenicol, tetracycline resistance; 'Tc', 'Cm', parts of cleaved gene.

degrees, the *recA_{Sm}* being slightly more effective than the *recA_{Pm}* (Figs 2a and b). It is concluded that (i) the RecA proteins of *S. marcescens* and *P. mirabilis* are active in *E. coli* and that (ii) both are also negatively complemented by the chromosomal *recA1_{Ec}* (*recA1* from *E. coli*) as is the cloned wild-type allele of *E. coli*.

In a strain deleted for the *recA* and *recBCD* genes (*E. coli* JV3) the *recA* and *recBCD* genes from the same or from different species were combined by double transformations. Since pNE1 and pDV11 or pDV31 are not compatible, strains with spontaneous cointegrates of these plasmids were isolated on plates containing antibiotics selecting for both plasmids. The cointegrated plasmids were named pSS1 (*recA_{Sm} recBCD_{Sm}*) and pPS2 (*recA_{Pm} recBCD_{Sm}*) (Table 1) and were shown by restriction analysis to contain the complete cloned fragments with the *recA* and *recBCD* genes, respectively (data not shown). The restoration of UV resistance

Fig. 2. UV resistance of *E. coli* strains with combined heterologous *recA* and *recBCD* genes. The two letters in parentheses indicate the origin of *recA/recBCD* genes present in the strains (E, *E. coli*; S, *S. marcescens*; P, *P. mirabilis*). A dash indicates the absence of genes. (a) Strains in which *E. coli* and *S. marcescens* genes are combined, and *recBCD* deletion strains; (b) strains in which *P. mirabilis* genes are combined with *E. coli* or *S. marcescens* genes; (c) *recA* deletion strains. The curves are means of two or three experiments.

conferred by the *recA* plus *recBCD* genes coming from the same species decreased in the order *E. coli* > *S. marcescens* > *P. mirabilis* (Figs 2a and b). Remarkably, restoration by the two *P. mirabilis* enzymes [JV3(pDV31)(pPG35)] was higher than when the two components came from different species, such as *recA_{Pm} recBCD_{Sm}* [JV3(pPS2)] or *recA_{Sm} recBCD_{Pm}* [JV3(pDV1)(pPG35)] (Fig. 2b). A similar advantage of

Fig. 3. Recombination proficiency of *E. coli* strains with *recA* and *recBCD* genes from *E. coli*, *S. marcescens* and *P. mirabilis*. The recombination frequencies in Hfr crosses using the strains listed above as recipients were performed and evaluated as described in Methods. The origin of the *recA* and *recBCD* genes is indicated by capital letters (E, *E. coli*; S, *S. marcescens*; P, *P. mirabilis*). A dash indicates the absence of genes. The recombination frequencies in strains without *recA* genes (not included in the figure) were lower than the limit of detection (0.3%), irrespective of the presence or absence of *recBCD* genes.

an intraspecies over several interspecies combinations was also observed in recombination studies (see next section). [In the strain JV3(pDV1)(pPG35) plasmid pDV1 containing *recA*_{Sm} was used instead of pDV11 to select double transformants with pPG35, which has identical antibiotic resistance genes to pDV11 (Table 1). Plasmids pDV1 and pDV11 conferred identical UV resistance upon an *E. coli* *recA1* mutant (data not shown)].

In those strains missing either *recA* or *recBCD* genes, the UV resistance decreased depending on the origin of the residual recombination enzyme again in the order *E. coli* > *S. marcescens* > *P. mirabilis* (Figs 2a and c).

Restoration of recombination proficiency by heterologous *recA* and *recBCD* genes

The relative recombination frequencies determined in 66 conjugation experiments with 12 recipient strains are summarized in Fig. 3. In the *recA* deletion mutant *E. coli* JV12, the activity of *recA*_{Sm} (located on pDV11) produced 90% of the *E. coli* wild-type level of recombination, whereas *recA*_{Pm} (on pDV31) provided only 47%. A similarly decreasing order of recombination frequencies was obtained in strains with the *recBCD* genes of *S. marcescens* instead of the *E. coli* *recBCD* genes and with the *recA* gene from *E. coli* (100%), *S. marcescens* (87%) or *P. mirabilis* (53%).

Strains carrying the *recBCD* genes of *P. mirabilis* showed generally reduced recombination proficiencies compared to the *E. coli* wild-type. The recombination frequencies were highest with the *recA* gene of *P. mirabilis* and lower with *recA* of *E. coli* or *S. marcescens*. Similar to what was observed in the UV resistance studies, there was a recombinational advantage of the strain with the *recA* and *recBCD* genes of *P. mirabilis* over strains carrying interspecies combinations of the genes, i.e. the combination *recA*_{Pm} *recBCD*_{Pm} was more efficient than the combinations *recA*_{Ec} *recBCD*_{Pm}, *recA*_{Sm} *recBCD*_{Pm}, *recA*_{Pm} *recBCD*_{Ec}, and *recA*_{Pm} *recBCD*_{Sm} (Fig. 3, see Discussion).

Recombination in strains lacking *recBCD* genes was low and followed the same decreasing tendency as reported for UV resistance, i.e. *recA*_{Ec} > *recA*_{Sm} > *recA*_{Pm} (Fig. 3). Recombination in strains without a *recA* gene was below the limit of detection (0.3%), irrespective of the presence or absence of *recBCD* genes.

Taken together, there is a high correlation of recombination proficiency and UV resistance for all 16 strains examined having all possible combinations of the *recA* and *recBCD* genes from three enteric bacteria. Both phenotypes decreased stepwise upon replacement of *E. coli* *recA* plus *recBCD* by the corresponding genes of *S. marcescens* and *P. mirabilis*. Even lower degrees of repair and recombination were obtained with several interspecies combinations.

Discussion

The *recA* genes from *S. marcescens* and *P. mirabilis* cloned into single-copy vectors are sufficiently expressed to restore near wild-type levels of recombination proficiency and UV resistance to a *recA* deletion mutant of *E. coli*. Both are partially recessive to the *recA1* allele of *E. coli*. This is concluded from the fact that the UV resistance conferred upon *E. coli* Δ *recA* significantly exceeded the UV resistance conferred upon *E. coli* *recA1*. This is in agreement with previous observations on negative complementation of RecA protein by the RecA1 polypeptide in *E. coli* (Yancey & Porter, 1984; Sedgwick & Goodwin, 1985). It indicates that like wild-type *E. coli* RecA protein, the RecA proteins of *S. marcescens* and *P. mirabilis* form heteropolymers with the *recA1* mutant polypeptide *in vivo*.

It has already been reported that the *E. coli* *recBCD* genes can be replaced by gene groups isolated from *P. mirabilis* and *S. marcescens* (Weichenhan & Wackernagel, 1988; McKittrick & Smith, 1989; Rinken *et al.*, 1991). *In vivo* several hybrid enzymes composed from subunits of *E. coli*, *S. marcescens* and *P. mirabilis* formed and exhibited varying levels of recombinational activity, although the restriction maps of the *recBCD* genes from the three species were quite different (Weichenhan & Wackernagel, 1988; Rinken *et al.*, 1991).

The *recA* and *recBCD* genes of *S. marcescens* or *P. mirabilis*, when simultaneously replacing the equivalent *E. coli* genes, promoted conjugational recombination and repair of UV damage. This suggests the existence of pathways of recombination in *S. marcescens* and *P. mirabilis* similar or identical to the RecBCD pathway of *E. coli*. These pathways are expected to recognize Chi sequences as hot spots of recombination because the respective RecBCD enzymes do that in *E. coli* (Weichenhan & Wackernagel, 1988, 1989; McKittrick & Smith, 1989). The observed recombination proficiencies conferred by intraspecies pairs of *recA* plus *recBCD* genes in *E. coli* had the order *E. coli* > *S. marcescens* > *P. mirabilis* (Fig. 3) and resembled the decrease of the repair capacities (Fig. 2).

An important result of this work was the observation that the potential of recombination (and repair) of several interspecies enzyme combinations was even lower than the lowest value obtained with the 'parental' intraspecies combinations. Specifically, the interspecies combinations *recA_{Ec} recBCD_{Pm}*, *recA_{Sm} recBCD_{Pm}*, *recA_{Pm} recBCD_{Ec}*, and *recA_{Pm} recBCD_{Sm}* all failed to reach the level obtained with the intraspecies combination of the two *P. mirabilis* enzymes (Fig. 3). The lower efficiencies cannot result from an insufficient expression of the *P. mirabilis* genes or a low activity of the *P. mirabilis* RecA protein or RecBCD enzyme produced in

E. coli, because together they achieved a higher level of recombination than any of the interspecies combinations. Also, the genes of *E. coli* and *S. marcescens* were expressed sufficiently, because they, when combined with genes from the same species, produced high levels of recombination. Thus, if not the activity of the single enzyme components, what is the reason for the hampered recombination in interspecies systems? We suggest that the efficiency drop (in recombination and repair) of interspecies combinations reflects a decrease or the absence of a cooperation between RecA protein and the RecBCD enzyme which normally occurs between these enzymes of the same species. Presumably, this cooperation ensures efficient repair and recombination. It probably relies on species-specific protein-protein interactions. This is in agreement with the fact that bacterial RecA and RecBCD enzymes, although functionally similar and structurally related, have species-specific primary structures and/or biochemical properties (Akaboshi *et al.*, 1989; Ball *et al.*, 1990; Zhao & McEntee, 1990; Weichenhan & Wackernagel, 1989; Rinken *et al.*, 1991).

The indication of a cooperation between the RecA protein and the RecBCD enzyme obtained in this study does not point to the mechanism of cooperation at work. However, on the basis of current models of the RecBCD pathway of recombination (Mahajan, 1988; Smith, 1988; Thaler & Stahl, 1988) and the biochemical activities of RecA protein and RecBCD enzyme one can envisage cooperative interactions between these enzymes at various steps during the recombination process. Formation of D-loops and the initiation of Holliday junctions are both catalysed by RecA protein (Radding, 1989). The processing of D-loops through cleavage by RecBCD enzyme has been demonstrated (Wiegand *et al.*, 1977; DasGupta *et al.*, 1979). Also the resolution of Holliday junctions is speculated to be catalysed by RecBCD enzyme (Thaler & Stahl, 1988; Taylor & Smith, 1990). If so, these reactions could involve an interaction of RecBCD enzyme with RecA protein either bound to single-stranded DNA in the D-loop or still present in the heteroduplex nucleoprotein filament, and this could stimulate recombinant formation (Radding, 1989). Similarly, if the RecBCD enzyme is involved in branch migration, as has been proposed by Kowalczykowski & Roman (1990), interactions between RecBCD and RecA proteins could facilitate recombination also by adjusting the high rate of DNA unwinding by RecBCD enzyme to the slow RecA-promoted strand transfer (Roman & Kowalczykowski, 1989*a, b*). An example of a species-specific interaction of recombination proteins has been provided by studies which showed the inability of RecA_{Pm} protein to catalyse strand transfer in the presence of *E. coli* SSB protein and that the ATPase

activity of RecA_{Pm} but not of RecA_{Ec} protein is blocked by *E. coli* SSB protein (West *et al.*, 1983). Further studies aimed at confirming the proposed cooperation of RecA and RecBCD enzymes by *in vitro* experiments are necessary.

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