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Cloning of the *recB*, *recC*, and *recD* Genes from *Proteus mirabilis* in *Escherichia coli*: In Vivo Formation of Active Hybrid Enzymes

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We cloned chromosomal DNA fragments from *Proteus mirabilis* which complement *recBCD* deletion mutants of *Escherichia coli* by restoring (i) recombination proficiency in conjugation, (ii) normal resistance to UV irradiation, and (iii) ATP-dependent exonuclease activity for duplex DNA. The data indicate that the order of the genes *thyA*, *recC*, *recB*, *recD*, and *argA* is similar in both *P. mirabilis* and *E. coli*. Hybrid enzymes formed in vivo were active in repair and recombination.

ATP-dependent exonuclease activities (also termed exonuclease V [exoV]; *recBCD* enzyme) (1, 20) have been detected in many bacterial species (17, 20). The exoV of *Escherichia coli* consists of three different subunits coded by genes *recB*, *recC*, and *recD* (1, 2). This enzyme has several activities in vitro which are stimulated by or depend on ATP hydrolysis (13, 15, 19). Mutations in *E. coli recB* or *recC* genes abolish exoV activity, decrease recombination proficiency in conjugation, and render cells sensitive to DNA damage (4); whereas mutations in *recD* reduce exonucleolytic degradation of duplex DNA (1). The *E. coli recBCD* genes have been cloned (5, 11, 16; G. Romanowski, D. Weichenhan, M. Gram, and W. Wackernagel, Life Sci. Adv. Ser. C, in press) and sequenced (7–9). To examine the functional, biochemical, and genetic relatedness of bacterial exoV enzymes, we cloned the *recBCD* gene region of *Proteus mirabilis*. The strains used in this study are described in Table 1.

In *E. coli* the genes *thyA*, *recC*, *recB*, *recD*, and *argA* lie in close proximity on the chromosome (1, 5) (Fig. 1). We assumed that the arrangement was similar on the *P. mirabilis* chromosome. By complementation of the *argA* and *thyA* deletion mutations of *E. coli* V360 Δ(*argA-recB*)*231* and V364 Δ(*recC-thyA*)*238* (3), we identified the plasmid pPG3, which bore the *P. mirabilis argA* gene ($argA_{Pm}$), and plasmids pPG5 and pPG6, each of which bore $thyA_{Pm}$ (Fig. 1). These plasmids were isolated from a genomic library from *P. mirabilis* PG1300 prepared by cloning 10-kilobase-pair *Sau*3A fragments into pBR322. From the location of the single *Kpn*I sites in pPG3 and pPG5, we hypothesized that $thyA^+$ and $argA^+$ might be located on one *Kpn*I fragment. Cloning of about 20-kbp *Kpn*I fragments from *P. mirabilis* PG1300 into vector pJRD184 (10) and complementation of the *E. coli thyA* mutant WA690 resulted in the identification of pPG35 and pPG53, each of which contained $thyA_{Pm}$ on identical 18.5-kilobase-pair *Kpn*I fragments, but in opposite orientations. Restriction analysis revealed that these plasmids overlap with major parts of pPG3 and pPG5 (Fig. 1).

The presence of the *P. mirabilis recB*, *recC*, and *recD* genes on the pPG plasmids was verified through complementation tests with *E. coli recBCD* deletion mutants. UV irradiation (21) and determination of exoV activity (measured as ATP-dependent degradation of duplex DNA) (22) were performed as described previously. Conjugations were performed by standard procedures. *E. coli* strains that lacked a functional *recB* or *recC* gene are sensitive to UV irradiation (20). Wild-type UV resistance was restored in WA676 Δ(*argA-recB*)*231* by pPG3 (Fig. 2A) and in WA675 Δ(*argA-thyA*)*232* by pPG35, pPG53, and by the simultaneous presence of both pPG3 and pPG5 (Fig. 2B; pPG53 not shown). In contrast, WA675 with pPG3, pPG5, or pPG3 plus pPG6 (data not shown), as well as WA677 Δ(*recC-thyA*)*238* with pPG5, exhibited only slightly increased UV resistance (Fig. 2A and B). Restoration of conjugational recombination proficiency (Table 2) was observed for WA675 with pPG35 and with pPG3 plus pPG5. Strain WA676 was complemented to 40% by pPG3. No complementation was obtained in WA677(pPG5). The exoV activity in crude extracts was 48 U/mg of protein in *E. coli* wild-type WA674(pBR322). WA675(pPG35) had about 25%, WA676(pPG3) had about 19%, and WA675(pPG3 plus pPG5) had only about 7% of this activity. In strain WA677 (pPG5), no exoV activity was measurable. These results indicate that *P. mirabilis* genes $recB_{Pm}$, $recC_{Pm}$, and $recD_{Pm}$ are present on pPG35 (and pPG53) and that $recB_{Pm}$ and $recD_{Pm}$ are present on pPG3. Complementation of WA675 by pPG3 plus pPG5 (Fig. 2B; Table 2) revealed that $recC_{Pm}$ must be present on pPG5. However, pPG5 did not complement the *recC* deletion mutant WA677. Since pPG351 also did not complement WA677 (Fig. 1 and 2A), it is concluded that pPG5 encodes an intact and not a truncated $recC_{Pm}$ subunit which forms active enzyme only with the $recB_{Pm}$ and $recD_{Pm}$ subunits, provided that expression is sufficient. Locations of the *P. mirabilis* genes were determined with deletion derivatives of pPG35 and pPG53 (Fig. 1). A phage T4 2^- mutant plates on $recB^+$ $recC^+$ $recD^+$ strains with low efficiency but plates well on *recD* mutants (2). On the *E. coli recD* mutant V220, the plating efficiency was decreased by the presence of pPG311 by a factor of about 60, placing $recD_{Pm}$ on the 2.2-kilobase-pair *Bgl*II-*Sma*I fragment (Fig. 1).

Reciprocal complementation of the *P. mirabilis* exoV mutant PG674N12 with respect to exoV activity and UV resistance was also achieved with pDW1 (14a), which carries the *E. coli recBCD* genes (data not shown). Thus, mutual expression of DNA occurs in both organisms and extends the observation of interspecies substitution of the *recA* protein in *E. coli* and *P. mirabilis* (6).

Southern hybridization between the cloned *recBCD* regions from *P. mirabilis* and *E. coli* did not reveal sequence relatedness under conditions of high stringency (data not shown). In addition, no similarities were observed between the restriction patterns (Fig. 1).

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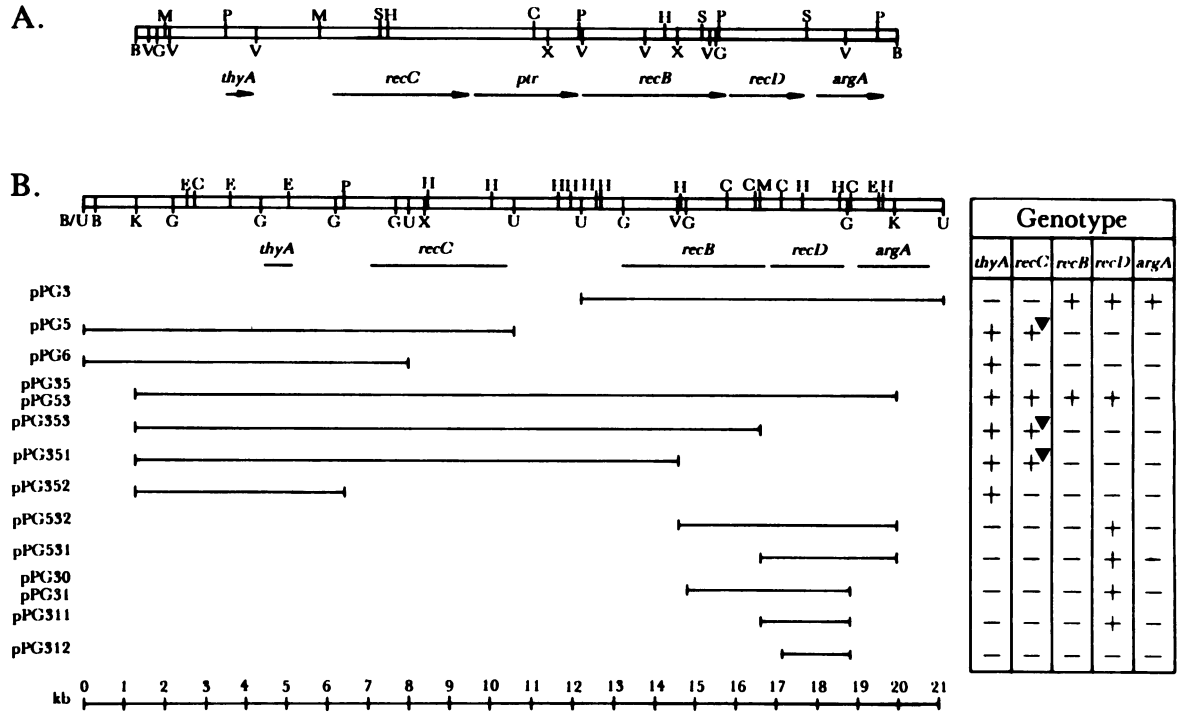

FIG. 1. Correlation of the physical and genetic maps of the *recBCD* region of the *E. coli* (A) and *P. mirabilis* (B) chromosomes. (A) The location, extent, and direction of transcription of the *E. coli* genes are shown according to published data (1, 5). (B) The location and extent of the genes were deduced from complementation analyses and from the extents of their *E. coli* counterparts by using the position of $recD_{Pm}$ as a fixed point; only relevant *Sau*3A sites are indicated; subcloned fragments used in the complementation analyses are shown below the physical map. In the genotype box, arrowheads indicate that genotypes were phenotypically detectable only in connection with pPG3 expressing $recB_{Pm}$ and $recD_{Pm}$. Restriction enzyme abbreviations: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; M, *Sma*I; P, *Pst*I; S, *Sal*I; U, *Sau*3A; V, *Pvu*II; X, *Xho*I. kb, Kilobase pairs.

Results of this study have shown that exoV of *P. mirabilis* is composed of subunits corresponding to proteins encoded by the *recB*, *recC*, and *recD* genes of *E. coli* and that the relative and vicinal order of genes *thyA*, *recC*, *recB*, *recD*, and *argA* in the *P. mirabilis* genome is similar to that in *E. coli* (Fig. 1). Similar observations were recently made with the cloned *recBCD* gene region from *Serratia marcescens* (unpublished data). Subunit substitution between *E. coli* and *P. mirabilis* indicates the in vivo assembly of *recBCD* enzyme subunits from different species into functional hybrid enzymes. Two hybrid enzymes of the composition $recB_{Pm}$ *recC* of *E. coli* ($recC_{Ec}$) $recD_{Pm}$ [in WA676(pPG3)] and $recB_{Ec}$ $recC_{Ec}$ $recD_{Pm}$ [in V220(pPG311)] were functionally active in *E. coli*. The enzyme of type $recB_{Ec}$ $recC_{Pm}$ $recD_{Ec}$ [in WA677(pPG5)] either did not form or was inactive. The structural similarities between at least two of the exoV subunits and heterologous functioning of the complete exoV enzymes from *E. coli* and *P. mirabilis* and of *recA* and

TABLE 1. Bacterial strains used in this study

Strain	Relevant genotype	Source or reference
E. coli		
K-12		
WA690	*recA1 hsdR17* (r_K^- m_K^+) *thyA*[a]	This work
WA674	*recB*$^+$ *recC*$^+$ *recD*$^+$ *lacZ*::Tn*10 proA2*[b]	This work
WA675	Δ(*argA-thyA*) *232 lacZ*::Tn*10 proA2*[b]	This work
WA676	Δ(*argA-recB*) *231 lacZ*::Tn*10 proA2*[b]	This work
WA677	Δ(*recC-thyA*) *238 lacZ*::Tn*10 proA2*[b]	This work
V220	*recD1011*	1
P. mirabilis		
PG1300	exoV$^+$	R. Rieger
PG674N12	exoV$^-$	R. Rieger

[a] Isolated by trimethoprim selection of *E. coli* DH5 (D. Hanahan).

[b] Obtained by conjugal transfer of *lacZ*::Tn*10 proA2* with BT38 (HfrH *thi-1 rbs-1 lacZ*::Tn*10 proA2*) into K-12s (R. Devoret), V186, V360, and V364 (3) for strains WA674, WA675, WA676, and WA677, respectively.

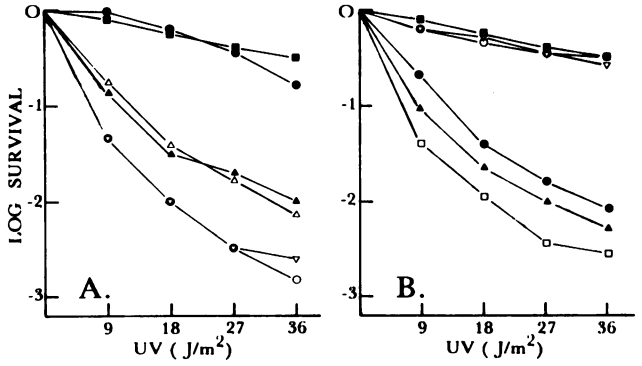

FIG. 2. UV sensitivity of transformed *recBCD* deletion mutants and the wild-type strain WA674 of *E. coli*. (A) WA674 (*recB*$^+$*recC*$^+$ *recD*$^+$) with pBR322 (■); WA676 Δ(*argA-recB*)*231* with pPG3 (●) or pBR322 (○); WA677 Δ(*recC-thyA*)*238* with pPG5 (▲), pPG351 (△), or pBR322 (▽). (B) WA674 (*recB*$^+$*recC*$^+$*recD*$^+$) with pBR322 (■); WA675 Δ(*argA-thyA*)*232* with pPG3 and pPG5 (○), pPG35 (▽), pPG3 (●), pPG5 (▲), or pBR322 (□).

TABLE 2. Effect of pPG3, pPG5, and pPG35 on the recombination proficiency of *E. coli recBCD* deletion mutants

E. coli strain and genotype	Recombination proficiency (relative frequency of recombinants/viable recipient) with[a]:	
	Vector	Recombinant plasmid
WA674 *recB*$^+$ *recC*$^+$ *recD*$^+$	pBR322 (1)	
WA675 Δ(*argA-thyA*) *232*	pJRD184 (0.04)	pPG35 (1)
WA675 Δ(*argA-thyA*) *232*	pBR322 (0.05)	pPG3 plus pPG5 (0.35)
WA676 Δ(*argA-recB*) *231*	pBR322 (0.01)	pPG3 (0.4)
WA677 Δ(*recC-thyA*) *238*	pBR322 (0.01)	pPG5 (0.01)

[a] The donor was HfrH strain WA213 (A. L. Taylor). Selection was for *pro*$^+$ (Ap^r) exconjugants. The recombinant frequency for WA674(pBR322) was 1.4×10^{-2} per viable recipient cell. The recombination frequencies were normalized to the F'128 transfer from WA493 Δ(*pro-lac*)*XIII* F'128 *pro*$^+$ *lac*$^+$ (B. Bachmann) to each of the recipient strains tested.

lexA proteins (6, 12, 14, 18) indicate considerable evolutionary conservation of basic enzymes of macromolecular DNA metabolism.

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