NOTES

Isolation and Characterization of the *Vibrio cholerae* *recA* Gene

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A 3.6-kilobase *Pst*I fragment was isolated from a *Vibrio cholerae* chromosomal DNA library and shown to encode RecA-like activity in complementation studies with *Escherichia coli* *recA* mutants. Although DNA hybridization experiments failed to detect any homology between the *E. coli* and *V. cholerae* *recA* genes, hyperimmune antiserum produced against purified *E. coli* RecA protein recognized epitopes shared by the *V. cholerae* protein. The *V. cholerae* chromosomal fragments, when cloned and transferred to *E. coli*, provided the missing *recA* functions, including (i) resistance to the alkylating agent methyl methanesulfonate, (ii) resistance to UV irradiation, and (iii) promotion of homologous recombination in Hfr mating experiments.

The *recA* gene product from *Escherichia coli* is a multifunctional protein involved in the regulation of several essential cellular functions, including homologous recombination (18) and resistance to UV and X-irradiation, as well as to other DNA-damaging reagents (3). The RecA protein possesses a proteolytic activity which cleaves the LexA repressor and results in expression of the SOS regulatory response (11, 12). Purified *E. coli* RecA protein is a single polypeptide of 37,842 daltons (20). The *Salmonella typhimurium* RecA protein has a similar molecular mass and displays similar functional activities; however, the proteins are distinguishable on the basis of characteristic antigenic determinants and proteolytic digestion patterns (17). RecA protein-like activities have been identified in several other species, including *Streptococcus faecalis* (25), *Proteus mirabilis* (5), *Shigella flexneri* (9), *Rhizobium meliloti* (1), *Erwinia carotovora* (9), and *Pseudomonas aeruginosa* (16). Immunochemical evidence suggests the presence of conserved functional domains among many of these RecA proteins, in spite of a lack of measurable homology in their DNA coding sequences (9).

In this study, a DNA fragment containing the *Vibrio cholerae* CA401 *recA* gene was identified by use of interspecies complementation of *recA* lesions in *E. coli*. The *V. cholerae* *recA* gene restored resistance to alkylating agents and UV irradiation and increased recombination frequencies in the *E. coli* mutant background. These observations confirm a study by Goldberg and Mekalanos (6), published during the revision of this report, in which these authors used a similar approach to isolate the *recA* gene from *V. cholerae* 569B. The cloned gene was inactivated by insertional mutagenesis, and the lesion was recombined into the *V. cholerae* chromosome, resulting in phenotypic characteristics similar to those of *E. coli* *recA* lesions. In addition, the *V. cholerae* RecA protein was implicated in amplification and deletion events associated with *ctx*, the structural gene for cholera toxin (7). We extended these observations to show that the *V. cholerae* RecA protein shared common epitopes detected by antiserum against the purified *E. coli*

protein. As in other species, the functional characteristics of the *V. cholerae* RecA protein were conserved in spite of considerable DNA sequence divergence.

A *V. cholerae* gene bank in *E. coli* HB101 (13) was obtained from J. B. Kaper, Center for Vaccine Development, Baltimore, Md. The bank was constructed by cloning *V. cholerae* chromosomal DNA, partially digested with *Sau*3A, into the *Bam*HI site of the cosmid vector pH79. To examine interspecies complementation of the *recA* lesion, strain HB101 cells containing this gene bank were grown overnight on Luria broth (15) plates with carbenicillin (50 µg/ml), pooled, diluted to a concentration of about 10⁴ cells per ml, and plated on Luria broth plates supplemented with 0.01% methyl methanesulfonate (MMS). Plasmid DNA was extracted (2) from MMS-resistant colonies and used to transform strain HB101 to carbenicillin resistance. These HB101 transformants were also MMS resistant. One such plasmid, designated pVR1, was examined by restriction endonuclease digestion analysis and shown to contain a 15-kilobase (kb) *V. cholerae* chromosomal DNA fragment. A collection of subclones was obtained by digestion of pVR1 with various enzymes, ligation into appropriate sites in pBR328, and transformation of HB101. From these subclones, the MMS resistance phenotype was localized to a 3.6-kb *Pst*I fragment, and the recombinant plasmid containing this fragment was designated pVR3. A preliminary restriction map of the 3.6-kb *Pst*I fragment was constructed (Fig. 1.) Subclones containing the 2.7-kb *Pst*I-*Bam*HI fragment (pVR4) or the 0.9-kb *Bam*HI-*Pst*I fragment (pVR5) were unable to confer MMS resistance when introduced into strain HB101, indicating that the *V. cholerae* gene spans the *Bam*HI site in pVR3.

To determine whether the *V. cholerae* DNA fragment which conferred MMS resistance to HB101 was functionally analogous to the *E. coli* *recA* gene, the ability of this fragment to complement other defects associated with *recA* mutations was examined. *E. coli* X7700 *recA* (15) was transformed to carbenicillin resistance with pVR1, and all transformants were MMS resistant. In addition, pVR1 suppressed the UV sensitivity phenotype associated with the *recA* mutation (data not shown). The presence of the recombinant plasmid resulted in a 10³- to 10⁵-fold higher level of survival when the strain was exposed to UV irradiation than

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FIG. 1. Partial restriction nuclease map of pVR3 and its derivatives. The *V. cholerae* chromosomal DNA fragment from the recombinant plasmid pVR3, which expresses RecA⁺ activity, is depicted, along with subclones which have lost the activity. -----, Approximate location of the *recA* gene. B, *Bam*HI; H, *Hind*III; Hc, *Hinc*II; P, *Pst*I; S, *Sal*I.

that seen in the strain without the plasmid, although the level was somewhat lower than the resistance level of the *E. coli* parent strain X7700. pVR1 also suppressed the UV sensitivity of *E. coli* JC10284, which contains a deletion of the entire *recA* gene (4). Similar results were obtained when pVR3 was introduced into X7700 *recA*; however, neither pVR4 nor pVR5 were able to suppress the UV sensitivity phenotype. Since the *E. coli* RecA protein is multifunctional and plays a role in recombination between homologous DNA sequences, the proposed *V. cholerae* *recA* gene was examined for its ability to facilitate genetic recombination in Hfr mating experiments. The presence of the plasmid in the *E. coli* *recA* mutant strain (X7700 *recA*) increased the frequency of Pro⁺ Str^r recombinants in a standard Hfr mating experiment by 1000-fold, to a frequency approaching that of the RecA⁺ parent (data not shown).

The extent of homology between the primary DNA sequences of these *recA* genes was tested by Southern blotting with either the *E. coli* or the *V. cholerae* *recA* gene as a probe. The 3.6-kb *Pst*I fragment from pVR3 was isolated from a low-melting-temperature agarose gel (13), nick translated (19), and hybridized to *Pst*I-digested genomic DNA, isolated by the procedure of Marmur (14) from *E. coli*, *V. cholerae*, and *S. typhimurium*. The genomic DNA digests were transferred to nitrocellulose paper (24) and hybridized to the *recA* probe for 16 h at 56°C in a solution containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt reagent (1× Denhardt reagent is 0.2% Ficoll plus 0.2% polyvinylpyrrolidone plus 0.2% bovine serum albumin), 200 µg of denatured salmon sperm DNA per ml, and 0.5% sodium dodecyl sulfate. The filters were washed exhaustively in 3× SSC at 46°C, dried, and autoradiographed. Under these low-stringency conditions, there was no detectable DNA hybridization between the *V. cholerae* probe and *E. coli* chromosomal DNA; however, a weak hybridization signal was detected with the *S. typhimurium* genome (Fig. 2A). Similarly, when a 3.0-kb *Bam*HI fragment from pBEU14 (23) containing the *E. coli* *recA* gene was used to probe *Bam*HI-generated genomic fragments from these species, there were no detectable signals with either the *V. cholerae* or *S. typhimurium* chromosome (Fig. 2B).

Antigenic similarities between the *E. coli* and *V. cholerae* RecA proteins were examined with polyclonal hyperimmune antiserum raised against the purified *E. coli* protein. It has been shown previously that the *in vivo* synthesis of the RecA protein can be induced to a greater level by the presence of nalidixic acid at a concentration (60 µg/ml) lower than that

required to inhibit DNA synthesis (23). *E. coli* *recA* mutant strains carrying *recA* genes on a multicopy plasmid were induced to produce high levels of RecA protein under these conditions. *E. coli* HB101 was transformed with pVR2 (a recombinant plasmid composed of a 3.0-kb *Bam*HI fragment from pBEU14 containing the *E. coli* *recA* gene inserted into the *Bam*HI site of pBR328), pVR3 (containing the *V. cholerae* *recA* gene), or the pVR3 subclones, pVR4 and pVR5, which have no functional RecA activity. Transformants were induced with nalidixic acid and lysed, and their protein products were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5 to 20% gradient gel

FIG. 2. Hybridization of the *E. coli* and *V. cholerae* *recA* genes to the chromosomal digests of different bacterial strains. Chromosomal DNA was isolated and digested with the designated restriction endonuclease, and the DNA fragments were separated on 0.7% agarose gels and transferred to nitrocellulose (24). (A) Autoradiograph of the Southern blot of the gel with the ³²P-labeled 3.6-kb *Pst*I fragment containing the *V. cholerae* *recA* gene as a probe. Lanes: 1, Positions of *Hind*III-cleaved lambda DNA size markers; 2, *Pst*I-digested *V. cholerae* CA401 DNA; 3, *Pst*I-digested *E. coli* MC4100 DNA; 4, *Pst*I-digested *S. typhimurium* DNA. (B) Autoradiograph of the Southern blot of the gel with the ³²P-labeled 3.0-kb *Bam*HI fragment containing the *E. coli* *recA* gene as a probe. Lanes: 1, Positions of *Hind*III-cleaved lambda DNA markers; 2, *Bam*HI-digested *V. cholerae* CA401 DNA; 3, *Bam*HI-digested *S. typhimurium* DNA; 4, *Bam*HI-digested *E. coli* MC4100 DNA.

by the procedure of Laemmli (10) modified to include 0.5 M urea (21). The proteins were transferred to nitrocellulose paper as described by Towbin et al. (22).

Rabbit anti-RecA (*E. coli*) antiserum, diluted 1:2000 in 0.9% NaCl–10 mM Tris (pH 7.4)–3% bovine serum albumin, was incubated with the nitrocellulose blot for 16 h at 4°C in the same buffer. The paper was washed and incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories, Malvern, Pa.) and developed with 4-chloro-1-naphthal as chromagen (8). The antiserum recognized the purified *E. coli* RecA protein against which it was produced, as well as the *E. coli* RecA protein induced from pVR2 (Fig. 3). In addition, cross-reactivity with the *V. cholerae* RecA protein encoded by pVR3 was clearly evident, although the *V. cholerae* protein migrated slightly more slowly than its *E. coli* counterpart.

This report describes the isolation and characterization of a genomic DNA fragment containing the *V. cholerae* *recA* gene identified by interspecies complementation of *E. coli* *recA* mutations. It confirms and extends the observations recently reported by Goldberg and Mekalanos (6). The presence of a recombinant plasmid containing this fragment in *E. coli* *recA* derivatives allowed the expression of the following phenotypes: (i) resistance to alkylating agents such as MMS, (ii) resistance to UV irradiation, and (iii) normal recombination capabilities in Hfr mating experiments. This approach was similar to that used to isolate *recA* genes from *R. meliloti* (1), *Pseudomonas aeruginosa* (16), *Proteus vulgaris* (9), *Erwinia carotovora* (9), and *Shigella flexneri* (9). The *V. cholerae* *recA* gene was localized to a 3.6-kb *Pst*I fragment, and subcloning evidence suggests that it spanned the single *Bam*HI site in that fragment. The interspecies complementation data presented in this report and that of Goldberg and Mekalanos (6) provide evidence that the *E.*

coli and *V. cholerae* RecA proteins are functionally analogous.

In vivo induction of *V. cholerae* *recA* gene expression from this recombinant plasmid in the presence of nalidixic acid resulted in the overproduction of a protein with a molecular weight of about 40,000, detected by antiserum against the purified *E. coli* RecA protein. This apparent size agreed with that reported for the *V. cholerae* RecA protein expressed from a recombinant plasmid in *E. coli* minicells (6). The present observation extends previous immunochemical evidence which suggests the presence of conserved antigenic structures among RecA proteins from a variety of species (9). However, in spite of the structural and functional relatedness of these analogous proteins, DNA hybridization experiments with their structural genes suggest considerable diversity in the primary DNA sequence. The *E. coli* *recA* gene does not hybridize (under low-stringency conditions) to the *Proteus vulgaris* (9), *Erwinia carotovora* (9), *S. typhimurium*, or *V. cholerae* *recA* genes but shows considerable homology to the *R. meliloti* (1) and *Shigella flexneri* (9) *recA* genes. The *V. cholerae* and *S. typhimurium* genes also shared detectable homology. These observations suggest that there is overall DNA sequence diversity among this group of related genes; however, the functional complementation and immunochemical evidence supports the hypothesis that the DNA (and therefore amino acid) sequences of the functional moieties of these proteins are conserved. The varying efficiency with which these heterologous RecA proteins are capable of functional interspecies complementation suggests that mutations which have altered functionally important amino acids have also accumulated to some degree.

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FIG. 3. Immunological cross-reactivity between the *V. cholerae* and *E. coli* RecA proteins. Cellular extracts were prepared from nalidixic-acid-treated cells, electrophoresed, transferred to nitrocellulose paper, and incubated with the anti-RecA (*E. coli*) antiserum. Lanes: 1, HB101; 2, HB101(pBR328); 3, HB101(pVR2); 4, HB101(pVR3); 5, purified *E. coli* RecA protein. The arrow identifies the position of the purified *E. coli* RecA protein.

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