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CLONING OF uvrA, lexC AND ssb GENES OF ESCHERICHIA COLI

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SUMMARY We have constructed a recombinant plasmid carrying a DNA fragment of the E. coli chromosome that specifically complements the uvrA, lexC and ssb mutations of this bacterium. Preliminary experiments indicate that this complementation is due to the presence of the structural genes on this plasmid.

INTRODUCTION

Nucleotide excision repair in E. coli is a complex reaction involving several steps and a number of genes (1). Among these genes, uvrA, uvrB and uvrC (2) are thought to be involved in the incision of damaged DNA (3). The enzymology of incision is poorly understood because it has proved difficult to purify the uvr proteins. To aid in the purification, we have been engaged in cloning these genes (4). In this report we describe the construction of a recombinant plasmid that complements uvrA mutations. This plasmid was found to also complement lexC (5) and ssb, the gene coding for the E. coli single strand-binding protein (6) which may be allelic with lexC (6).

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains used in this study are listed in Table 1. All are E. coli K-12 derivatives with the exception of H508 which is a hybrid of E. coli strains K-12 and C and PAM2611 which is a K-12 strain into which the lexC113 allele from a B strain was transduced. The plasmids used were pBR322 (11), pDR2000 (this work) and its insertional derivative pDR2044.

Enzymes and restriction analysis. Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs and were used as recommended by the supplier. Plasmid DNAs digested with either one or two restriction enzymes were analyzed on 0.7% agarose slab gels. EcoRI digested P22 bacteriophage DNA (12) was used as a molecular weight standard.

Cloning of the uvrA gene. Total cellular DNA (200 μg/ml) from the E. coli strain CGSC4260 which is diploid for the uvrA gene and pBR322 plasmid DNA

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TABLE 1

Bacterial Strains Used in This Study

Strain	Sex	Relevant Genotype	Reference or Source
CSR603	F-	uvrA6 recA1 phr-1 rpsL31 nalA	Sancar and Rupert (7)
AB1889	F-	uvrA19	*CGSC1889
H508	F-	uvrA103	CGSC4952
152Δ	F-	recA1 Δ(uvrB) (uvrB deletion)	D. Freifelder (8)
DR1984	F-	uvrC34 recA1	This laboratory
KLF12/JC1553	F112	recA1	CGSC4260
MG1063	F+	recA56	Guyer (9)
AB2494	F-	lexA1	CGSC2494
DM511	F-	tsl-1	D. Mount (10)
PAM2611	Hfr	lexC113	B. Johnson
KLC436	F-	ssb-1	J. Chase
NH5168	F-	ssb-1 (Thy+ derivative of KLC436)	P. Howard-Flanders

* CGSC denotes E. coli Genetic Stock Center, Yale University

(50 μg/ml) were digested with EcoRI, mixed in a 1 to 1 ratio, ligated and CSR603 was transformed with the recombinant DNA. The transformed culture was incubated in Luria broth (13) for 3 hr, diluted 1/10 into Luria broth containing 10 μg/ml tetracycline and incubated at 37°C overnight. The cells were collected by centrifugation, washed with and resuspended in $M9_2$ buffer (14), and irradiated with an ultraviolet (254nm) fluence of 2.5 J/m^2. Cells were collected again, resuspended in Luria broth and were grown to stationary phase; UV irradiation was repeated and 0.1 ml samples of the irradiated culture were plated on Luria agar supplemented with 10 μg/ml tetracycline. Single colonies were picked from these plates and were tested for UV sensitivity (2).

Isolation of plasmids with γδ insertions. Guyer (9) has recently reported that F mediated transfer of pBR322 results in the insertion of the γδ sequence of F into pBR322 at random sites. We used this technique to insert the γδ sequence into the uvrA plasmid as follows: MG1063 carrying pDR2000 and CSR603 were both grown in Luria broth to a cell density of 2 x 10^8 cells/ml. They were mixed together and were incubated at 37°C for 90 min with gentle shaking. 0.1 ml samples were plated on Luria agar containing streptomycin (50 μg/ml), nalidixic acid (50 μg/ml) and tetracycline (20 μg/ml) to select for CSR603 cells that received insertional derivatives of pDR2000. 480 single colonies were picked from these plates and were tested for UV sensitivity by irradiating with 2.5 J/m^2 to determine whether the derivatives of pDR2000 with the inserts caused the cells to become more resistant to UV as is the case with pDR2000 itself, or to retain the sensitivity of CSR603 that occurs if expression of the uvrA gene on the plasmid is blocked by insertion of γδ.

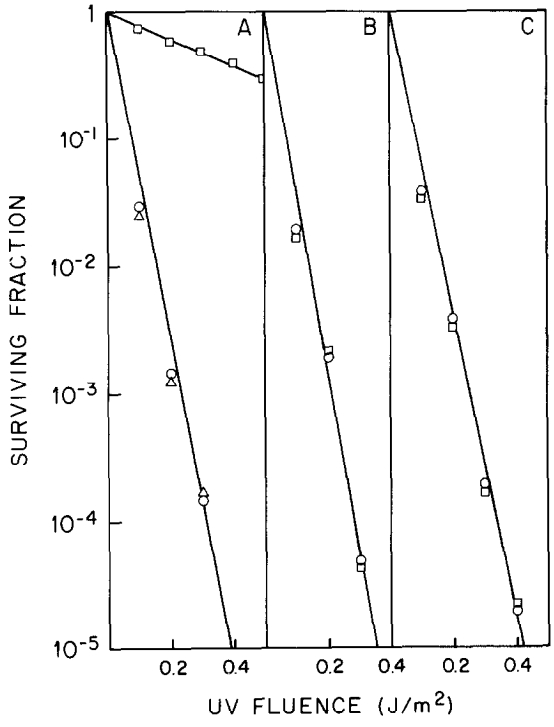

Fig. 1 Effect of the plasmid pDR2000 on the survival of strains carrying different uvr alleles. The strains used were: A, CSR603uvrA6; B, 152Δ (uvrB deletion); C, DR1984uvrC34. O, no plasmid; □, pDR2000; Δ, pDR2044. The cells also carried the recA1 allele and were irradiated in stationary phase.

RESULTS

Cloning and insertional inactivation of the uvrA gene. 0.5 ml of recombinant DNA mixture was divided into 5 aliquots and each was used to transform CSR603 (recA1 uvrA6 phr-1) selecting for UV-resistant cells as described in Materials and Methods. After the second UV irradiation, two of the cultures showed UV-resistance similar to that of uvrA recA cells. Single colonies were isolated from these cultures and grown up for the preparation of plasmid DNA. One class of the UV-resistant cells contained pBR322 and these cells were presumed to be revertants of the uvrA gene. The other class contained a plasmid of 13.7 kilobase pairs which simultaneously transformed CSR603 cells to Uvr^+ and Tet^R Amp^R phenotypes at the frequency of about 10^5 transformants/μg DNA. This plasmid, pDR2000, also complemented the uvrA19 and uvrA103 alleles of the uvrA gene but did not change the sensitivity of 2 strains carrying either a uvrB or uvrC mutation (Figure 1).

For further studies on the expression of pDR2000, we thought that it would be helpful to have a derivative in which the uvrA gene was inactivated by the introduction of an insertion sequence. Since the method developed by Guyer for pBR322 (9) appeared applicable to pBR322 derivatives with segments of the bacterial chromosome, we used a similar approach to obtain plasmids with γδ insertions. Of the 480 insertional derivatives of pDR2000 tested as described in Materials and Methods, 10 failed to complement the uvrA gene. In Figure 1 we present the effects of one of these plasmids, pDR2044, on the UV survival of CSR603. As is evident from the figure, the insertion of γδ in this plasmid completely prevents the expression of the uvrA gene.

Complementation of lexC by pDR2000. The lexA and lexC genes are within approximately 0.2 min distance from uvrA on the E. coli genetic map (lexA, 90.35; lexC, 90.75; uvrA, 90.55) (5). Therefore it was possible that pDR2000 might carry one of the lex genes in addition to the uvrA gene.

To test for lexA, AB2494 was transformed with pDR2000 and the transformants were tested for UV sensitivity. As can be seen from Figure 2A, pDR2000 has only a slight effect on the survival of the lexA strain AB2494. However, since it is known that the mutant lexA allele is dominant over the wild-type allele when both are present as single copies (15), this result did not conclusively eliminate the possibility that lexA was present on pDR2000. Another lexA mutation, tsl-1, which makes lexA cells temperature sensitive for cell division, is recessive to wild type (10). We transformed DM511 (tsl-1) with pDR2000 and found no effect of the plasmid on the temperature sensitivity of this strain (data not shown). These results taken together led us to conclude that pDR2000 did not carry lexA.

In contrast, pDR2000 conferred UV-resistance to both PAM2611 (lexC) (Figure 2) and NH5168 (ssb-1) (data not shown) indicating that the plasmid carried the lexC and ssb genes. This interpretation was further strengthened because pDR2044, the plasmid with the insertion that inactivates uvrA, still complements lexC (Fig. 2) showing that the complementation of uvrA

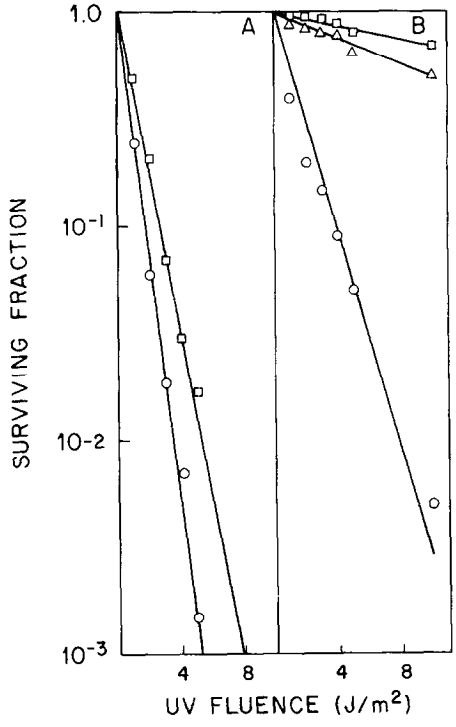

Fig. 2 Effect of the plasmid pDR2000 on the survival of strains carrying lexA1 and lexC113. The strains used were: A, AB2494lexA1; B, PAM2611 lexC113. O, no plasmid; □, pDR2000; Δ, pDR2044. The cells were irradiated in stationary phase.

and lexC occur through different gene products and that the complementation of lexC cannot be explained by phenotypic suppression due to the overproduction of uvrA protein.

Restriction map of pDR2000. The uvrA-lexC plasmid was digested with the restriction enzymes EcoRI, Pst I, BamHI and HindIII and analyzed on 0.7% agarose gels. From these digests, the restriction map shown in Figure 3 was constructed. An interesting feature of this map is the presence of an EcoRI site in the chromosomal fragment. Since EcoRI was used for cloning, we suspect that our original digestion of chromosomal DNA with EcoRI was partial.

DISCUSSION

We have isolated a recombinant plasmid that complements the uvrA, lexC and ssb mutations of E. coli. The specificity of this complementation indicates that the plasmid carries these structural genes. The availability

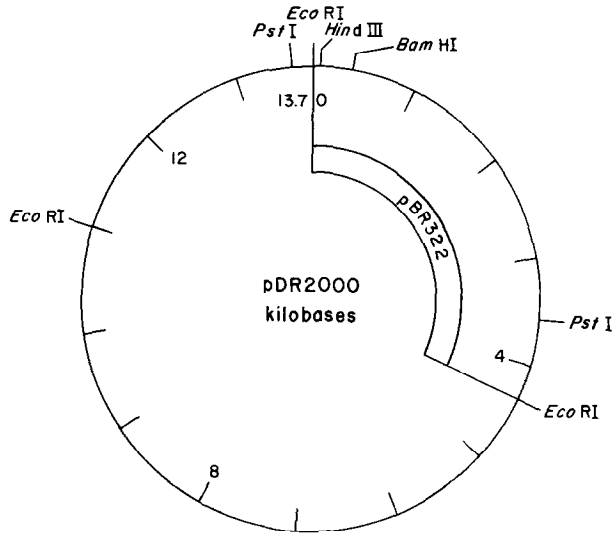

Fig. 3 Restriction map of pDR2000 showing the sites cut by EcoRI, BamHI, Pst I and HindIII.

of this plasmid should help in the identification and purification of the uvrA protein. We have preliminary evidence showing that the uvrA gene codes for a single polypeptide with a Mr of about 100,000 (Sancar, Seltzer and Rupp, unpublished data).

The gene coding for the single strand DNA binding protein, ssb, has been identified and mapped recently by Meyer, Glassberg and Kornberg (6) who suggested that the lexC gene may be the same as the ssb gene. Our results are consistent with this suggestion but do not prove it. Tests now in progress to isolate and characterize insertions in the lexC and ssb genes should show conclusively whether or not they are the same gene. Although a method exists for the large scale purification of ssb protein (16), the availability of a multicopy plasmid carrying the gene should make purification easier.

Since lexC113 is known to be dominant over the wild type allele (17), it might seem surprising that pDR2000 complemented the lexC113 mutation. However, this may simply be a statistical consequence of the lexC native protein being a tetramer as it is already known that the native ssb protein is composed of 4 ssb subunits (16). Assuming random association of subunits,

only 1 of 16 tetramers would have 4 wild type subunits starting from an equal mixture of wild type and lexC113 subunits (as might occur in a normal heterozygote), while a 9 to 1 ratio of wild type to lexC113 subunits (that might result from having the wild type allele present on a multicopy plasmid) would result in over half of the tetramers being made up entirely of wild type subunits. This difference might well be sufficient to overcome the dominance of the mutant allele observed in the earlier experiments.

Finally, we want to point out that the presence of uvrA, lexC and ssb on a chromosomal fragment 9 kilobase pairs in size puts an upper limit on the distance between these genes.

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