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Biochemistry

Identification and radiochemical purification of the *recA* protein of *Escherichia coli* K-12

(specialized transducing phages/phage protein labeling/gel electrophoresis/column chromatography)

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ABSTRACT **The product of the *recA* gene of *E. coli* has been identified by labeling proteins synthesized in UV-treated cells after infection with specialized transducing phages carrying the *recA* gene. Following infection of UV-treated cells by λ*precA*, which carries the *recA*$^+$ gene, a major protein with a molecular weight of 43,000 is detected on polyacrylamide gels containing sodium dodecyl sulfate. This protein is also made after infection of suppressing hosts by λ*precA99*, which carries an amber *recA*$^-$ mutation, but is not synthesized after infection of nonsuppressing hosts by this transducing phage. A spontaneous *recA*$^+$ revertant of λ*precA99* induces synthesis of this protein after infection of a nonsuppressing host. The product of the *recA* gene is a soluble protein found in a complex with a molecular weight of approximately 150,000 after mild detergent lysis of cells.**

Homologous recombination in *Escherichia coli* is absolutely dependent upon the function of the *recA* gene. Mutations in this locus reduce homologous recombination by five orders of magnitude (1) and result in other severe physiological effects. Most prominent among these is the extreme sensitivity of *recA*$^-$ mutants to agents which damage DNA: UV, x-irradiation, and alkylating agents such as mitomycin C and nitrofurantoin (2–4). Most *recA*$^-$ mutants have very low rates of spontaneous prophage induction (1) and no UV-stimulated prophage induction (1, 5). UV-induced mutagenesis is absent in *recA*$^-$ mutants (6), while both spontaneous and UV-induced DNA degradation are elevated in *recA*$^-$ cells (1, 7). The product of the *recA* gene is inferred to be a protein, because amber (8), temperature sensitive (9), and cold sensitive (K. McEntee, unpublished results) *recA*$^-$ mutations have been isolated.

The products of several other genes affecting recombination and repair have been determined. An enzyme with exo- and endonucleolytic functions, exonuclease V, was identified as the product of the *recB* and *recC* genes (10–12). Furthermore, an analysis of mutations which restore recombination to *recB*$^-$ and *recC*$^-$ mutants revealed the participation of several other genes in recombination, and led to the identification and biochemical characterization of their products (13–15). Nevertheless, the protein product of the *recA* gene has defied efforts to identify it.

Recently, we isolated plaque-forming specialized transducing phages for the *recA* region of the *E. coli* chromosome (16). These phages were obtained from λ lysogens in which the prophage is integrated in the *srl* locus, a group of genes involved in sorbitol metabolism (see Fig. 1). The transducing phages were used to infect UV-irradiated cells in order to preferentially label transducing phage proteins. By this method, we have identified the protein product of the *recA* gene of *E. coli*.

MATERIALS AND METHODS

Bacterial Strains and Bacteriophages. Suppressor-free strain 159 (*uvrA*$^-$ *strA sup*$^+$) and two derivative strains which carry amber suppressors (KM1591, *supD;* KM1593; *supF*) were used for the protein labeling experiments. The two suppressor strains were constructed by isolating a spontaneous *trp-tonB* deletion (17) in strain 159, and introducing *supF* by P1*kc* transduction or introducing *supD* by conjugaton with Hfr Hayes strain DW103 (*supD strA*$^+$) and selecting *trp*$^+$ *strA* recombinants. The Sup$^-$ recombinants were identified by the ability to plate amber mutants of phage T7. λ*c*I857 lysogens were picked from single plaques, recloned, and tested for λ immunity.

The three different transducing phages used in this work and a diagram of the *srl-recA* region of *E. coli* showing the regions carried by these phages are presented in Fig. 1. The λ*precA*, λ*precA99*, and λ*precA99R* phages all carry the *srlC* and *recA* genes, but do not carry a functional *alaS* gene, based upon their inability to complement mutations in this locus. The λ*precA* and λ*precA99* phages were isolated from independent Srl$^-$ lysogens and may not carry the same amount of bacterial DNA. λ*precA99* carries the amber *recA99* mutation. λ*precA99R* is a spontaneous *recA*$^+$ revertant of λ*precA99*, selected to restore UV light resistance to a Sup$^+$ *recA*$^-$ host. The λ*psrl* phage carries less bacterial DNA than the *recA* phages; it complements *srl-1* and other mutations in *srlC*, but does not complement any *recA* mutation (K. McEntee, unpublished results). The λ*dalaS* phage was obtained from the same secondary site lysogen as λ*precA*, and was selected for its ability to complement mutations in *alaS*. This phage also transduces the *srlC* and *recA* genes (K. McEntee, unpublished results).

All transducing phages used carry the temperature sensitive *c*I857 mutation and are not UV-inducible (*ind*$^-$). Phage stocks were prepared by thermal induction of the appropriate transducing phage lysogen and were purified by buoyant density centrifugation in CsCl as described by Miller (17).

UV Irradiation of Cells, Phage Infection, and Gel Electrophoresis. The procedure for phage protein labeling is essentially that of Ptashne (18), as modified by Hendrix (19). Logarithmic phase cultures of cells in K115 medium (20) containing 0.2% glycerol (wt/vol) and 0.2% maltose were irradiated with either a "low" dose of UV light (6000 ergs/mm^2) or a "high" UV dose (24,000 ergs/mm^2), with a 15 watt General Electric germicidal lamp, at a cell density of 2×10^8 cells per ml (1 erg is 10^{-7} joule). After irradiation, cells were incubated in the same medium in the dark for 60 min at 30°. Cells were then concentrated 5-fold in K115 medium containing 20 mM $MgSO_4$, 0.2% glycerol, and 0.1% maltose and chilled on ice. The appropriate phage was added at a multiplicity of 5–10 and allowed to adsorb for 10 min. Then L-[*U*-^{14}C]isoleucine, (1 μCi, 330 mCi/mmol) was added to 4 ml of cells and the suspension was shaken at 30°. Incorporation was allowed to continue for 60–90 min. At the end of the labeling period, the cells were collected by centrifugation, washed with 20 mM Tris·HCl at

Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

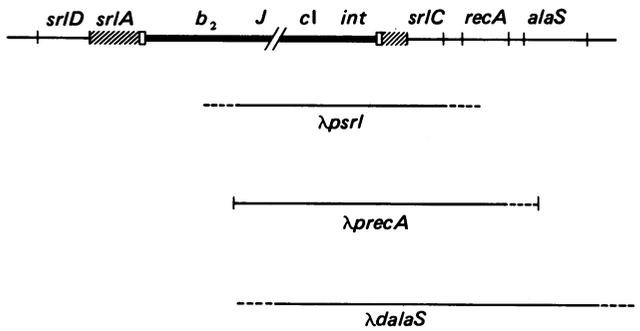

FIG. 1. A schematic representation of the *srl-recA-alaS* region of the *E. coli* chromosome that shows the location of λ integration in *srlA* and the structure of the transducing phages used in this work. The orientation of λ in *srl* and the structure of λ*precA* have been reported (27). The order of the three *srl* genes and the assignment of the site of λ integration to the *srlA* cistron are based on genetic mapping, complementation studies, and enzymology (ref. 24; K. McEntee and J. E. Hesse, unpublished results). The genetic symbols are those of Bachmann *et al.* (24). The dotted lines represent uncertainty as to the ends of the bacterial substitutions on the transducing phages.

pH 7.5 containing 1 mM $MgCl_2$, and resuspended in electrophoresis buffer containing sodium dodecyl sulfate ($NaDodSO_4$) (21). Labeled proteins were analyzed by slab gel electrophoresis in 20% acrylamide containing $NaDodSO_4$ (21) followed by treatment with 2,5-diphenyloxazole for gel fluorography as described (22).

RESULTS

Heavily UV-irradiated cells lose the capacity to incorporate labeled amino acids into proteins. The reduction in incorporation of exogenous amino acids is due to diminished transcription of the damaged chromosome. When an intact phage genome is introduced into these cells, amino acids are preferentially incorporated into proteins coded by the phage. This technique has been extremely useful in identifying phage proteins, as well as in analyzing the products of bacterial genes carried on specialized transducing phages (19, 23). We have used this technique to analyze the proteins made after infection by specialized *recA* transducing λ derivatives.

When strain 159 is irradiated with a high UV dose, infection with λ*precA* stimulates incorporation of radioactive isoleucine into protein by a factor of 3–5 (data not shown). Polyacrylamide gel analysis of λ*precA*-infected cell extracts indicates that a significant fraction of the label incorporated is found in a single protein band (Fig. 2A). This band is the largest protein incorporating significant amounts of label under these conditions. This protein is not detected after infection of the same nonsuppressing host with λ*precA99*, a phage which carries the amber *recA* mutation (Fig. 2B). These results suggest that the large protein labeled after λ*precA* infection is the product of the *recA* gene. This possibility was investigated by examining proteins made after λ*precA99* infection of strains KM1591 (*supD*) and KM1593 (*supF*). As shown in Fig. 3, infection of these suppressing strains results in the synthesis of a heavily labeled protein which migrates to the same position on the gel as the protein synthesized after λ*precA* infection. Densitometric analysis of these gel fluorographs indicates that this protein band is the only detectable difference between λ*precA99* infected Sup^+ (nonsuppressing) and Sup^- (suppressing) cell extracts. Because two amber suppressors, *supD* and *supF*, which suppress the *recA99* mutation *in vivo* restore the synthesis of the high-molecular-weight protein, we conclude that this protein is the product of the *recA* gene.

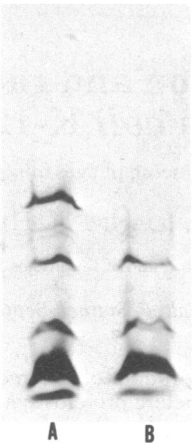

FIG. 2. Gel fluorograph of λ*precA* and λ*precA99* coded proteins. Strain 159 (Sup^+) was irradiated with approximately 24,000 ergs/mm^2, infected, labeled with L-[^{14}C]isoleucine for 60 min, and the proteins examined by $NaDodSO_4$/polyacrylamide gel electrophoresis as described in *Materials and Methods*. Approximately 100,000 cpm of each sample was applied to the gel; migration is from top to bottom. A, λ*precA*; B, λ*precA99*.

At the lower UV dose used in the experiment of Fig. 3, several proteins are labeled which are absent from gel patterns of infected cells which have received a high UV dose (see Fig. 2). These proteins are probably late proteins of phage λ; Hendrix has shown that late phage proteins are synthesized if the host cells receive a low dose of UV before infection, but not if a high dose of UV light is delivered (19). The dose of UV used does not affect the ability of the host to suppress the *recA99* mutation; the *recA* protein is made after λ*precA99* infection of strains KM1591 and KM1593 which have been irradiated with a high UV dose (data not shown).

Four additional experiments were performed to confirm the identification of this protein as the product of the *recA* gene: (*i*) related transducing phages which carry the *srlC* gene but do not complement *recA* mutations should not direct the synthesis of this protein after infection. The λ*psrl* phage isolated from the same secondary site lysogen as λ*precA* (see Fig. 1) is such a phage. The proteins induced by λ*psrl* are shown in Fig. 4B. No protein at the position of the *recA* band is detected on

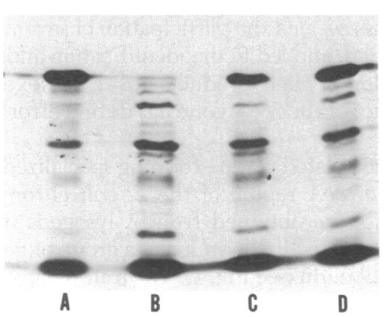

FIG. 3. Gel fluorograph of λ*precA99* proteins made in suppressing and nonsuppressing hosts. Samples were obtained and analyzed as described in Fig. 2, except that a UV dose of 6000 ergs/mm^2 was used. Approximately 10,000 cpm of each sample was applied to the gel; migration is from top to bottom. A, λ*precA* infection of strain 159 (nonsuppressing); B, λ*precA99* infection of strain 159; C, λ*precA99* infection of strain KM1591 (*supD*); D, λ*precA99* infection of strain KM1593 (*supF*).

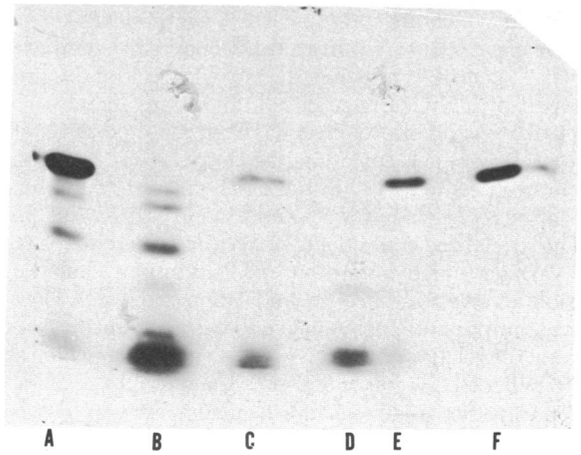

FIG. 4. Gel fluorograph of proteins induced by λ*precA*, λ*precA99*, λ*precA99R*, and λ*psrl*. Samples were prepared and analyzed as described in Fig. 3 except that protein labeling was performed for 90 min at 30°. Migration is from top to bottom. A, λ*precA* infection of strain 159; B, λ*psrl* infection of strain 159; C, λ*precA99* infection of strain KM1591 (λ); D, λ*psrl* infection of strain 159 (λ); E, λ*precA99R* infection of strain 159 (λ); F, λ*precA* infection of strain 159 (λ).

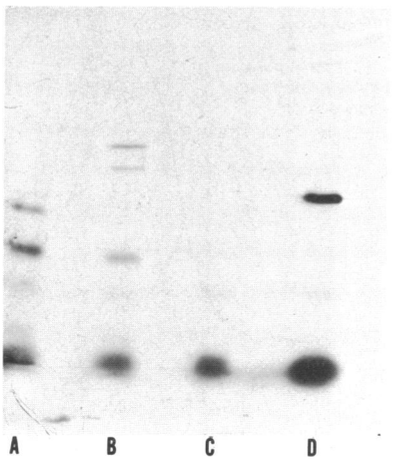

FIG. 5. Gel fluorograph of proteins made by λ*psrl*, λ, and λ*dalaS*. Samples of infected cell extracts were prepared and analyzed as described in Fig. 4. Migration is from top to bottom. A, λ*psrl* infection of strain KM1591; B, λ*c*I857 infection of strain KM1591; C, λ*psrl* infection of strain KM1593 (λ); D, λ*dalaS* infection of strain KM1593 (λ).

polyacrylamide gels. (*ii*) Because the *recA* gene is expressed in λ*precA* lysogens (16), the expression of the *recA* gene is not under phage repressor control. Therefore, the *recA* protein should be made after λ*precA* infection of a λ lysogen. To test this prediction, we used a λ*c*I857 *ind*$^-$ lysogen because this prophage is not induced by UV treatment of the host. As shown in Fig. 4F, the *recA* protein is made after infection of strain 159(λ*c*I857) by λ*precA*, but is not detected after infection of this host by λ*psrl* (Fig. 4D) or λ*precA99* (data not shown). The pattern of expression of the *recA* product in the infected lysogen is the same as found in the nonlysogenic hosts used above, but now the *recA* protein is the major radioactive species and represents greater than 80% of the label incorporated into proteins of molecular weight greater than 5000. With the elimination of synthesis of λ proteins in extracts of the infected lysogen, it is possible to discern at least one minor protein synthesized after infection of strain 159(λ*c*I857) by all the transducing phages (see below). This protein may be the product of the *srlC* gene which is carried by all the phages (see Fig. 1). We cannot exclude the possibility that this protein with a molecular weight of 15,000–20,000 is coded by a gene between *srlC* and *recA*, or that it is the product of the *srlA* gene fragment carried by the transducing derivatives. (*iii*) A *recA*$^+$ revertant of the λ*precA99* phage should direct synthesis of the *recA* protein after infection of a nonsuppressing host. Infection of Sup$^+$ strain 159(λ*c*I857) by the *recA*$^+$ revertant phage λ*precA99R* leads to isotopic labeling of the *recA* protein (Fig. 4E). Infection of this same host by the amber parental phage does not direct synthesis of this protein. (*iv*) Genetic mapping of the *recA* region indicates that the alanyl-tRNA-synthetase structural gene, *alaS*, is closely linked to *srlC* and *recA* (ref. 24; K. McEntee, in preparation). The gene order is shown in Fig. 1. A defective *alaS* transducing phage, designated λ*dalaS*, was isolated and used to infect UV-irradiated strain 159(λ*c*I857). Three proteins can be detected after infection of the λ lysogenic strain (Fig. 5D). The most heavily labeled band migrates to the same position as the *recA* protein. A high (greater than 70,000) molecular weight protein which is not induced by λ*precA* can also be detected on this gel. This protein may be the *alaS* gene product. The structure of the alanyl-tRNA synthetase of *E. coli* has been reported to be a dimer composed of identical subunits with a molecular weight of 70,000–90,000 (25).

Molecular Weight Estimate of the *recA* Protein. The molecular weight of the *recA* gene product was estimated by comparing the mobility of this protein on polyacrylamide gels with the mobilities of known λ proteins. The gel pattern of proteins made after λ infection is shown in Fig. 5B. Two high-molecular-weight proteins are synthesized after λ infection which are absent from gel patterns of λ*psrl*, λ*precA*, and λ*precA99* infected cells (see Fig. 3 and 4). Furthermore, these protein bands are absent from gel patterns of cells infected with phages carrying the *b*515 and *b*519 deletions (data not shown). These two deletions eliminate DNA from the *b*2 region of the phage chromosome (26). Hendrix has shown that proteins of molecular weight 56,000 and 48,000 are coded by genes in the *b*2 region and that these proteins are not made by phages which have a large *b*2 deletion or the smaller internal *b*515 and *b*519 deletions (19). The failure to detect these proteins in gel patterns of λ*precA* and λ*precA99* infected cells is consistent with the fact that the *srl-recA* substitution on these transducing phages is in the *b*2 region of the phage chromosome (ref. 27; K. McEntee, unpublished results). Based upon the molecular weight values of 56,000 and 48,000 for these *b*2 proteins, a plot of migration distance versus the logarithm of the molecular weight indicates that the *recA* monomer is approximately 43,000.

Radiochemical Purification of the *recA* Protein. Isotopic labeling of the proteins made after infection of a λ lysogen by λ*precA* leads to nearly radiochemically pure *recA* protein (see Fig. 4F). Such labeled extracts can be used to monitor purification of this protein by following the distribution of radioactivity during fractionation. After gentle detergent lysis and centrifugation at 48,000 × *g* for 25 min, approximately 90% of the radioactivity remains in the supernatant fraction. All of the radioactivity can be recovered in the supernatant after precipitation of the remaining DNA with 1% streptomycin sulfate. Chromatography of this streptomycin supernatant on Ultrogel AcA34 (LKB) results in a peak of radioactivity which elutes as a protein with a molecular weight of approximately 150,000 (Fig. 6). This molecular weight is consistent with a tetrameric form of the undissociated *recA* protein. Alternatively, the *recA* monomer might be complexed with other cell proteins. Purification of the protein to homogeneity will be required to distinguish between these possibilities.

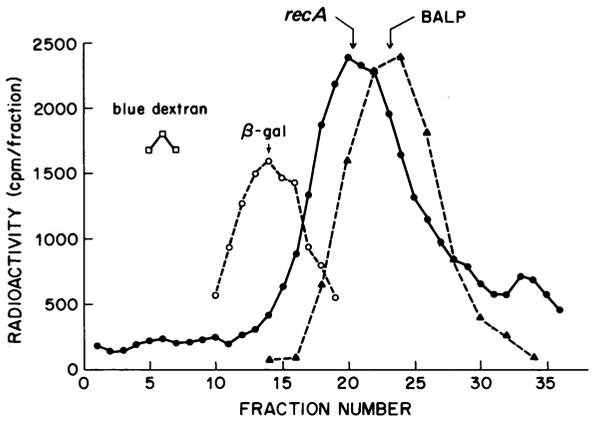

FIG. 6. Molecular sieve chromatography of radioactive *recA* protein. The radioactive sample was prepared by [^{14}C]isoleucine labeling of UV-irradiated strain 159 (λ) after infection with λ*precA* (see *Materials and Methods*). Labeled cells were lysed by the technique of Clewell and Helinski (31), and remaining nucleic acids were precipitated by the addition of 0.3 ml of 10% streptomycin sulfate. Greater than 90% of the trichloroacetic acid precipitable radioactivity remained in the supernatant and was in a single protein band as determined by gel electrophoresis. An 0.2 ml sample containing approximately 40,000 cpm of cell extract was applied to a 0.95 × 16 cm column of acrylamide-agarose (Ultrogel AcA34, LKB) and eluted in 10 mM Tris·HCl at pH 7.4, 5 mM $MgCl_2$, 0.2 M KCl, 10% glycerol (vol/vol), 50 μg/ml of bovine serum albumin, 0.5 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride. Fractions of 0.24 ml were collected and assayed for radioactivity. Blue dextran, bacterial alkaline phosphatase, (BALP), and β-galactosidase (β-gal) were added to the column for markers and were assayed as described (32). The addition of 1 mM phenylmethylsulfonyl fluoride was required to prevent degradation of the *recA* protein during purification. However, even in the presence of this protease inhibitor, a small amount of degradation to a 25,000–30,000 molecular weight fragment could be detected by gel analysis.

DISCUSSION

Protein labeling after infection by the specialized transducing phages λ*precA*, λ*precA99*, λ*psrl*, and λ*dalaS* has made possible the identification of a protein with molecular weight of 43,000 as the product of the *E. coli recA* gene. This identification is based upon the following observations: (*i*) this protein is a major protein synthesized after infection of irradiated hosts by λ*precA*; (*ii*) this protein is made after infection by λ*precA99* of *supD* and *supF* hosts, but is not made after infection of a nearly isogenic Sup$^+$ strain; (*iii*) this protein is not synthesized after infection by a specialized transducing phage which carries the nearby *srlC* gene but does not carry the *recA* gene; (*iv*) this protein is induced by λ*dalaS* which, by genetic tests, carries the *srlC*, *recA*, and *alaS* genes; (*v*) this protein is synthesized in Sup$^+$ hosts by a *recA*$^+$ revertant of λ*precA99*; and, (*vi*) this protein is made after infection of a λ lysogen by λ*precA* which indicates that the *recA* gene on this phage is not controlled by λ repressor and is probably transcribed from its own promoter. This last observation is consistent with the known properties of the *recA* transducing phages because the fully repressed prophages express the transduced bacterial genes (refs. 16 and 27; K. McEntee, unpublished results).

The *recA* protein is made abundantly after λ*precA* infection of UV-irradiated cells. One possible explanation for this overproduction is that UV damage induces synthesis of the *recA* protein. Induction of this protein might be required by the cell for repair of DNA lesions and regulation of cell division until segregation of repaired chromosomes can take place. At least one other protein, protein X, has been shown to be induced by treatments which abruptly halt DNA synthesis (28). The induction of protein X requires a functional *recA*$^+$ gene as well as a *lexA*$^+$ gene (29), although the biological role of this protein in repair of DNA damage or cell division is unknown. The molecular weight of protein X has been reported as 40,000 on polyacrylamide gels containing $NaDodSO_4$ (30). Experiments are in progress to ascertain the biochemical relationship between the *recA* gene product and protein X.

The specialized transducing phages described in this paper, and variants which carry other *recA* mutations, should make possible an investigation of the regulation of the *recA* locus and provide important biochemical and genetic information concerning the relation of the *recA* gene to mutations which affect UV repair, cell division, mutagenesis, and prophage induction. The purification and biochemical characterization of the *recA* protein should contribute to an understanding of these cellular processes as well as to the biochemical features of homologous recombination.

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