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Identification of a protein coded by pR plasmid and involved in SOS repair in E. coli

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ABSTRACT

The TP120 plasmid is known to determine enhanced UV survival in E. coli wild type and uvrB and PolA mutants but not in RecA mutant.

In order to analyze the function involved in the SOS repair, we have constructed a new plasmid named pR derived by cleavage of TP120 with Hind III endonuclease. This new plasmid maintains the Ap and UV resistance. The insertion of Tn5 transposon in the plasmid allows to select several pR::Tn5 plasmids whose UV resistance was inactivated by the transposition. The comparison of the protein synthesis in the minicells of the pR and pR::Tn5 shows that the pR codes for a 22.000 M.W. dalton protein which is absent in protein pattern of pR::Tn5.

INTRODUCTION

Several reports have indicated that certain plasmids are capable of reducing the ultraviolet (UV) sensitivity of the bacterial host containing them (1-3). In particular, the R factor TP120 (or R46) is known to increase the survival of UV in wild type, uvrB or polA mutants but not in recA mutant of E. coli (2,4). This observed dependance of TP120 on the recA function suggests that this plasmid might exert its effect by interaction with the recA-dependent SOS repair process (2). In E. coli this function is related with a complex group of inducible responses which seem to be coordinately regulated, dependent on recA and lexA genes, and are constütively expressed at high temperature in tif mutants (5,6).

TP120 and its natural derivative, pKM101, have the capacity to protect the cells from UV light as well as to enhance chemical mutagenesis (2), but they do not induce the synthesis of the recA protein, a peptide of 40.000 dalton (7,8). The

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synthesis of this protein is regulated by the lexA gene as well as being self regulated (8-10). The mechanism by which the TP120 enhances UV survival is not yet clear. Walker (2) has suggested that these plasmids amplify the activity of the cellular inducible error prone repair system by coding for one or more proteins that interact with some components of the cell's error prone repair.

In this paper we report the construction of a pR plasmid, derived from TP120, and the identification of a protein encoded by the pR plasmid, involved in SOS repair in E. coli C600.

MATERIALS AND METHODS

Bacterial strains and plasmid

We have used E. coli K12 C600 thr, leu, thi, lac-y, λ^S. The minicells producing strain was AR1062, a restrictionless derivative of P678-54. Plasmid TP120 is an antibiotic resistence, self-trasmissible plasmid of the N-incompatibility group, coding for resistance to tetracycline (Tc), ampicillin (Ap), streptomycin (Sm) and sulfonamide (Su) and capable of increasing UV survival in the bacterial host. TP120 plasmid and C600 were kindly supplied by E.M. Lederberger (Plasmid Reference Center, Dpt. of Medical Microbiology, Stanford University, Stanford, California). The pR plasmid (Ap^+, Uv^+) was constructed in our laboratory by cleavage of plasmid TP120 with Hind III restriction endonuclease.

UV irradiation

The source of UV light was a 30 W germicidal lamp emitting over 90% of its energy at 256 nm and shielded to give $5J.m^{-2}.sec^{-1}$. The fluences were measured by the use of a Latarjet UV-meter. To determine UV survival, 0.1 ml aliquots from appropriate dilutions of an overnight culture of bacteria in Luria broth were spread on L-agar plates. After the inocolum had dried, the plates were exposed to various doses of UV light. Colonies were counted after overnight incubation at 37°C.

Isolation of plasmids DNA

TP120 and its derivative pR plasmids (obtained as described later) were isolated according to Clewell and Helinsky (11) and purified by centrifugation to equilibrium in CsCl-ethidium brom-

ide gradients at 40.000 rpm in a 50Ti fixed angle Beckmann rotor.

Restriction enzyme digestion and gel electrophoresis of restricted DNA

The Hind III restriction endonuclease was purchased from New England Biolabs.

TP120 DNA and pR were digested to completation with Hind III restriction enzyme and DNA was electrophorized in 0.8% agarose gel in parallel with a λ DNA marker digested with the same enzyme.

Ligation and transformation

The Hind III digests of TP120 DNA (3 μg) was incubated in 10 μl of ligation buffer with T4 ligase (N.E. Biolabs.). The conditions of ligation were such as to favour the circularization rather than polymerization of the fragments according to Dugaiczyk et al.(12). The transformation was carried out according to Cohen et al. (13). The transformed bacteria were selected on agar plates containing ampicillin (20 μg/ml) and the bacterial colonies were tested for UV sensitivity.

Construction of pR::Tn5 plasmid

E. coli C600 strain harboring pR plasmid was infected with phage λ b221c1857 Tn5 (Kan) at a multiplicity of 5 phages/cell. After 15' adsorption at 30°C the cells were diluted 20-fold in L-broth and areated 30' at 30°C to permit the expression of the antibiotic resistance. Ap^+ Kan^+ transductans were selected on agar plates containing Ap (20 μg/ml) and Kan (30 μg/ml) (14).

Protein synthesis in minicells

The minicell-technique was used in order to analyze the protein synthesized by the plasmid. A fresh colony of AR1062 (+pR plasmid or +pR::Tn5) was suspended in 25 ml of M9 medium supplemented with 0.5% glucose, 1% vitamin free casaminoacids and ampicillin (20 μg/ml) for pR or ampicillin and kanamycin (30 μg/ml) for pR::Tn5. After 16hr growth at 37°C the bacterial culture was centrifuged 10' at 10.000 rpm in the Sorvall SS34 rotor and the bacterial pellet was resuspended in 0.5 ml BSG (0.85% NaCl, 0.03% KH_2PO_4, 0.06% Na_2HPO_4). Minicells were isolated and the proteins were purified according to Rambach and Hogness (15). The minicells were labelled by the addition of 50 μC of ^{35}S methionine (250 Ci/mmol) and incubated at 37°C for 60'.

Aliquots (2-5 µl) of the protein solution were layered onto a sodium dodecyl sulfate/polyacrylamide gel and electrophoresed at 18mA for 5-6 hrs at room temperature (16). The gels were dried, autoradiographs exposed to X-ray films for 12-24 hrs.

RESULTS

Construction of the pR plasmid

TP120 is a plasmid of molecular weight $3.3x10^7$ dalton, poorly studied in its structure and functions except for the specification of some character of antibiotic resistance. As we were interested in the identification of the protein(s) involved in the UV resistance function, we tried to construct a smaller viable plasmid which maintained the characters of antibiotic and UV light resistance.

The restriction enzyme Hind III digests TP120 DNA into two fragments of $\tilde{}15x10^6$ dalton M.W. and two smaller fragments respectively 1.5 and $0.8x10^6$ dalton (Fig.1). If one of these fragments contains the origin of replication as well as the genes responsible for antibiotic and UV resistance, it should be possible to circularize such DNA and to transform competent cells, thus obtaining a smaller plasmid which might be easier to analyze and handle. TP120 DNA was digested with Hind III restric-

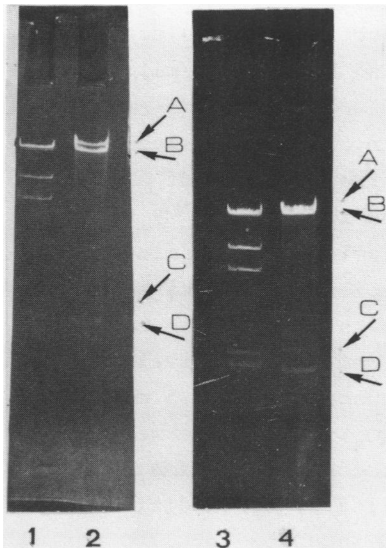

Fig.1. Hind III cleavage pattern of TP120. Purified TP120 DNA (3 µg) was digested with Hind III at 37°C in 60mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl buffer (pH 7.4). The digestion products were separated by electrophoresis through 0.8% agarose gel containing 35mM Tris, 1mM NaH_2PO_4 buffer (pH 7.8). The gel was stained with ethidium bromide (0.5 µg/ml).
(Track 1 and 3) λ DNA digested with Hind III (The sticky ends of λ were not melted).
(Track 2 and 4) TP120 DNA digested with Hind III. The molecular weights, determined by graphical interpolation, are approximately: $<15x10^6$ (A), $15x10^6$ (B), $1.5x10^6$ (C), $0.8x10^6$ (D)

tion enzyme and the resulting fragments were circularized by ligation with T4 ligase and used to transform E. coli C600 (see Materials and Methods).

We obtained a number of ampicillin resistant colonies which showed enhanced survival after UV irradiation and such resistance was the same as that of E. coli transformed by TP120 (Fig.2).

Electrophoretic analysis of the plasmid DNA on agarose gel revealed that these colonies were all transformed by the closed fragment of molecular weight $15x10^6$ dalton derived by TP120 (Fig. 3). Thus we have constructed a new plasmid, named pR, derived by restriction of TP120 and maintaining the regions for autonomous replication and for ampicillin and UV resistance.

Minicell-analysis of proteins encoded by pR plasmid

In order to identify the protein(s) coded by the region of pR plasmid conferring resistance to UV, we compared the electrophoresis pattern of the pR plasmid-coded proteins in E. coli minicells, with the pattern obtained from minicells in which the UV resistance had been inactivated by the insertion of Tn5 in pR.

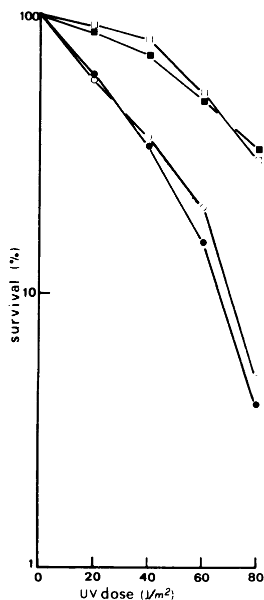

Fig.2. UV survival of C600 strain transformed with either TP120, pR or pR::Tn5 DNA. The transformants were selected on agar plates containing Ap, Tc, Sm, only Ap or Ap, Kan, respectively. The UV survival of the bacteria was determined as described in Materials and Methods.
● C600; ■ C600+TP120; □ C600+pR; ○ C600+pR::Tn5

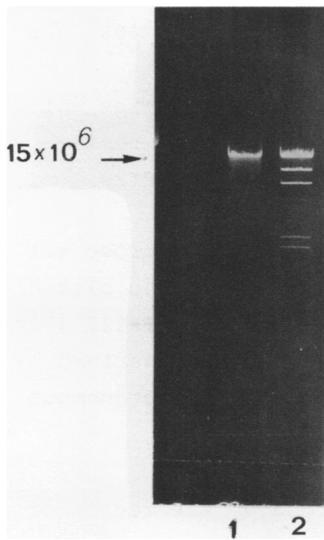

Fig.3. Identification of the Hind III fragment of TP120 plasmid (pR) which maintains the Ap-resistance gene and the UV-protective function. pR DNA (3 μg) purified from colonies grown on Ap agar plates was electrophoresed through a 0.8% agarose gel, as described in Fig.1: (track 1) pR plasmid digested with Hind III endonuclease ($15x10^6$ m.w.); (track 2) λ DNA digested with Hind III as molecular weight marker.

In fact it has been shown that the transposon Tn5, once inserted into a gene, eliminates all detectable gene function thus inducing mutation by insertion (14). We have selected therefore several ampicillin resistent pR::Tn5 colonies whose UV resistance was inactivated by transposition. The UV survival curves of C600 containing the pR::Tn5 plasmid show that the pR::Tn5 has lost the ability to protect the cells against killing by UV light (Fig.2).

The pR::Tn5 (Ap^+, Kan^r, UV^-) and pR (Ap^+, UV^+) plasmids were transferred to the restrictionless minicell producing strain AR1062. Minicells containing either pR or pR::Tn5 plasmids were labelled with ^{35}S methionine and isolated 8 hrs after stationary phase when the polypeptides synthesis of bacterial chromosome was absent (17). The labelled polypeptides were separated on SDS gel electrophoresis and detected by autoradiography. Comparison of the protein coded by pR and pR::Tn5 plasmids shows that the pR plasmid codes for a polypeptide of apparent 22.000 dalton molecular weight (Fig.4, track 2) which is absent in the protein pattern of the pR:Tn5 plasmid (Fig.4, track 1) whose UV resistance is inactivated by transposition. These data suggest that this protein is coded by the DNA sequences which were inactivated by transposition and might be involved in the process

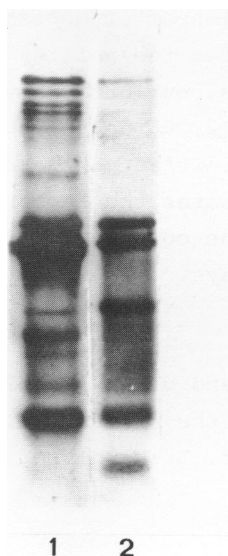

Fig.4. Gel electrophoresis of the polypeptides synthesized in minicells containing pR or pR::Tn5 plasmids. The arrow indicates the position of a polypeptide of M.W. about 22.000 which is present in pR (track 2) and absent in pR::Tn5 plasmid (track 1). The protein molecular weight markers were BSA (MW 68.000) and RNA polymerase subunits of 165.000, 155.000 and 39.000 respectively (not shown). The additional bands produced by pR::Tn5 and absent in the pR pattern are coded by the Tn5 transposon (19).

of UV resistance.

CONCLUSIONS

It is generally believed that the SOS repair response is normally repressed in bacteria and that the various functions belonging to it are triggered in response to a common regulatory signal emitted as a consequence of a specific block in DNA replication (18). Expression of SOS functions can be modified by mutation in either lexA gene or recA gene. Consequently, the products of these two genes are thought to regulate the SOS response. It has also been shown that plasmid TP120 enchances the UV resistance of $recA^{+}$ $lexA^{+}$ E. coli strains. The mechanism by which this effect is brought about has not been elucidated. However the observations that TP120 is unable to alter the UV response of $recA^{-}$ E. coli strains, which in turn are altered in SOS repair, suggests an interaction of the plasmid with these cellular functions (1).

In order to explore this hypothesis, we have constructed a new plasmid, pR, by cleavage of TP120. The pR plasmid maintains the resistance to ampicillin and the ability to enhance UV survival of E. coli C600 exhibited by the TP120 parent; our results

also show that pR codes for a protein of M.W. 22.000 dalton whose synthesis can be suppressed by the insertion of transposon Tn5. When this occurs, the plasmid (pR::Tn5) loses the ability to protect the cells against killing by UV. Thus, our data show a strong correlation between expression of the 22.000 dalton pR protein and the acquisition of resistance to UV. It seems therefore likely that the 22.000 dalton protein increases the repair activity in pR-containing cells by amplifying the constitutive level of cellular (recA, lexA) SOS repair pathways.

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