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Evidence for involvement of UvrB in elicitation of 'SIR' phenotype by *rpoB87-gyrA87* mutations in *lexA3* mutant of *Escherichia coli*

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ABSTRACT

An unconventional DNA repair termed SIR (SOS Independent Repair), specific to mitomycin C (MMC) damage elicited by a combination of specific Rif^R (*rpoB87*) and Nal^R (*gyrA87*) mutations in SOS un-inducible strains of *Escherichia coli* was reported by Kumaresan and Jayaraman (1988). We report here that the *rpoB87* mutation defines a $C_{1565}\rightarrow T_{1565}$ transition changing $S_{522}\rightarrow F_{522}$ and *gyrA87* defines a $G_{244}\rightarrow A_{244}$ transition changing $D_{82}\rightarrow N_{82}$. The reconstructed *lexA3 rpoB87 gyrA87* strain (DM49RN) exhibited resistance to MMC but not to UV as expected. When mutations in several genes implicated in SOS/NER were introduced into DM49RN strain, *uvrB* mutation alone decreased the MMC resistance and suppressed SIR phenotype. This was alleviated about two fold by a plasmid clone bearing the *uvrB*$^+$ allele. Neither SulA activity as measured based on filamentation and *sulA::gfp* fluorescence analyses nor the transcript levels of *sulA* as seen based on RT-PCR analyses indicate a change in *sulA* expression in DM49RN strain. However, *uvrB* transcript levels are increased with or without MMC treatment in the same strain. While the presence of *lexA3* allele in a plasmid clone was found to markedly decrease the MMC resistance of the DM49RN strain, the additional presence of *uvrB*$^+$ allele in the same clone alleviated the suppression of MMC resistance by *lexA3* allele to a considerable extent. These results indicate the increased expression of *uvrB* in the DM49RN strain is probably from the LexA dependent promoter of *uvrB*. The sequence analyses of various *uvrB* mutants including those isolated in this study using localized mutagenesis indicate the involvement of the nucleotide phosphate binding domain (ATPase domain) and the ATP binding domain and/or the DNA binding domain of the UvrB protein in the MMC repair in DM49RN. The possible involvement of UvrB protein in the MMC damage repair in DM49RN strain in relation to DNA repair is discussed.

1. Introduction

The DNA repair mechanisms in *Escherichia coli* that have been well understood and documented can be classified as the RecA independent, such as the *ada* response [1] or the RecA dependent [2] SOS response. It is well known and has been established over the years that the SOS mediated inducible repair system is under the control of the *recA* and the *lexA* genes [2]. On contact with single stranded DNA the RecA protein gets activated and mediates self-cleavage by LexA auto-protease [3,4]. This cleavage of LexA repressor results in activation of a cascade of genes. During initial stage of DNA damage, a set of genes including *uvrA*, *uvrB*, *etc.*, involved in nucleotide excision repair (NER) are expressed [4,5]. When this does not suffice, a second set of genes under tight LexA control including the *umuD*, *umuC*, *dinB*, *sulA*, *etc.*, are expressed which give rise to mutagenic repair [6,7]. Mutations in *recA* or *lexA* have been known to render the cell hypersensitive to DNA damaging agents. While the SOS mediated repair is general to many kinds of lesions [5], the other mechanisms identified are specific to certain specific types of lesions. The *ada* response is specific for methylated bases of purines or pyrimidines [8]. The repair of methylation at oxygen residues, O^6-MeG and O^4-MeT, caused by S_N1 agents such as N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and N-Methylnitrosourea (MNU) is mediated by the *ada* gene protein, an O^6-meG-DNA methyltransferase [9–11]. Another lesion specific SOS independent response reported is the UVM (UV Modulation of Mutagenesis) [12]. The UVM response is specific to 3,N^4-ethenocytosine (εC) [13] or εA [14] lesions caused by extrinsic carcinogens such as vinyl chloride and ethyl carbamate [15].

Mitomycin C (MMC) is a highly potent DNA damaging agent which causes DNA crosslinks and it has been shown that a single

Abbreviations: MMC, mitomycin C; Rif, rifampicin; Nal, nalidixic acid; SIR, SOS Independent Repair; tet, tetracycline; kan, kanamycin; cfu, colony forming units.

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crosslink per genome is sufficient to cause the death of a bacterial cell [16,17]. MMC, after reduction into vinylogous quinone methide, causes two N-alkylations in specific 5′-CpG-3′ regions in DNA which causes crosslinks [18]. Mitomycin C has been shown to be a potent inducer of the SOS response and the damage is repaired by the UvrABC dependent nucleotide excision repair [16]. The UvrB protein of the NER has been shown to play a major role in this as it interacts with all the components of the excision repair like the UvrA, UvrC, UvrD (helicase), DNA polymerase I and damaged DNA [19,20]. During the process of excision repair the UvrA and UvrB form a complex together which detects damaged DNA and using helicase activity inserts the β hairpin loop into the DNA helix followed by the release of UvrA to give the preincision complex [21]. UvrC then binds to the UvrB–DNA complex and causes incisions first at the 4th to 5th base in the 3′ side followed by a 5′ incision at 8th base upstream [22,23]. In related studies, mutant forms of UvrB have been shown to recognize damaged DNA on its own without UvrA [24] and homologue for the UvrC protein has also been identified named Cho [25].

Kumaresan and Jayaraman in 1988 [26] reported an unconventional DNA repair specific to MMC damage in SOS un-inducible strains and this repair was found to be elicited due to a specific combination of Rif^R (*rpoB87*) and Nal^R (*gyrA87*) mutations. The fact that this repair is seen in SOS uninducible strains led them to postulate that this repair could potentially be an unconventional SOS Independent Repair and hence the phenotype was termed as 'SIR'. MMC is believed to inhibit DNA synthesis and cause degradation of available DNA. Thus DNA degradation assays with *rpoB87 gyrA87* derivatives of *lexA3* Ind$^-$ strains which are SOS un-inducible but SIR proficient were carried out to analyse the levels of DNA damage recovery. This revealed that the DNA degradation was decreased to a considerable extent in this SIR$^+$ strain as compared to the parent *lexA3 rpoB*$^+$ *gyrA*$^+$ strain, DM49. Therefore, it can be said that the *rpoB87* and *gyrA87* mutations are able to overcome the MMC induced damage caused to DNA to significant levels. It was also shown that the SIR phenotype does not result due to deficiency in uptake of or reduction of mitomycin C because the prophage induction of mitomycin C in *recA*$^+$ λ lysogens was independent of *rpoB87* and *gyrA87* mutations. Therefore, it was proposed that the elicitation of the SIR$^+$ phenotype could perhaps be due to induction of a partially efficient DNA damage repair system/pathway by the combined effect of *rpoB87* and *gyrA87* mutations under SOS un-inducible conditions [26].

The elicitation of the proposed SIR phenotype is dependent on the mutant forms of β subunit of RNAP and GyrA subunit of DNA Gyrase apart from the RecA protein and the product encoded by the locus named *sir* which was found to map between 57 and 61 min [27] and possibly other unidentified gene(s). In this investigation, we have taken efforts to testify the view of SIR phenotype and functions involved in the same. We have shown here that *rpoB87* defines a C→T transition in the 522nd codon of *rpoB* and *gyrA87* defines a G→A transition in the 82nd codon of *gyrA*. Attempts taken to see the effect of *dinB::kan*, *umuDC::cat*, *uvrD::Tn5*, *uvrA::Tn10* and *ΔuvrB::Tn10kan* mutations in the elicitation of SIR phenotype clearly reveal that functional UvrB is mandatory for this unconventional (SIR) DNA repair. The data reported herein further validate that *sulA* transcription is not at all altered in SIR proficient *lexA3 rpoB87 gyrA87* strain but transcription of *uvrB* is increased irrespective of damage induction and might possibly be from the LexA dependent promoter of *uvrB*. We also believe our results indicate that the ATPase domain (domain 1a) and the ATP binding domain and/or the DNA binding domain (β hairpin) might play a role in the MMC resistance of the DM49RN strain.

2. Materials and methods

2.1. Bacterial strains used and construction

Given in Table 1 is the list of bacterial strains used in this study. Genetic nomenclature is according to Demerec *et al.* [28].

All P1 mediated transductions were performed as described in Miller (1972, 1992) [29,30]. The relevant recipient and donor strains are also mentioned in Table 1.

The *rpoB87* mutation was transduced from JK10AB using linked *argE*$^+$ marker and introduced into DM49 and the Arg$^+$ transductants obtained on selective minimal plates lacking arginine were screened for Rif resistant colonies. One such Rif resistant Arg$^+$ transductants was named DM49R and used for further experiments. The *gyrA87* mutation from JK10AB was linked with Tetracycline resistant marker *zfa723::Tn10* and the Tet^R transductants were screened for those transductants which retained *gyrA87*. This strain was named JK10ABT (*lexA3 rpoB87 gyrA87 zfa723::Tn10*). Using the P1 made on this strain, the recipient strains DM49 and DM49R were transduced for Tet^R colonies and the obtained Tet^R transductants were checked for acquisition of Nal resistance. One such Tet^R Nal^R transductants from DM49 and DM49R were named DM49N and DM49RN, respectively.

In order to enable mobilization of *uvrB* mutation to desired strains, the *uvrB* point mutations were first linked with kanamycin resistance marker *zbh3108::*Tn*10kan* by transducing the Kan^R marker into relevant *uvrB* mutants and looked for UV^S ones among the obtained Kan^R transductants. The *uvrB45* mutation in the strain AB2421 could not be directly linked to *zbh3108::*Tn*10kan* by screening for UV sensitivity because of the presence of *uvrA6* mutation which also affects UV sensitivity. Thus after transduction of *zbh3108::*Tn*10kan* marker into AB2421, P1 lysate made from few random transductants were transduced into the strain AB1157 and from those Kan^R transductants UV sensitive colonies were selected for further use. The respective *Tn10kan* linked *uvrB* mutants were then used as donor and the alleles were mobilized into the desired strains using P1 transduction.

2.2. Media and chemicals

LB and minimal media [29] with appropriate supplements were used. Cells were routinely grown in LB at 37 °C unless specified otherwise. Whenever required the following chemicals/antibiotics were added to the media in the final concentrations indicated. MMC (0.5 μg/ml), Rif (20 μg/ml), Nal (20 μg/ml), Tet (10 μg/ml), Kan (45 μg/ml), X-gal (30 μg/ml), and amino acids (30 μg/ml). The chemicals used were purchased from Himedia, India, Sigma, USA and Sisco Laboratories, India. MMC was purchased from Biobasic Inc., India. The primers used for the study were obtained from Chromous Biotech, Bangalore, India. The enzymes used were obtained from Fermentas, India.

2.3. Mitomycin C and UV survival assays

Overnight cultures were sub-cultured into fresh LB broth and grown till mid-log phase (~0.3 OD). Untreated samples were withdrawn at 0 min and were diluted. Appropriate dilutions were plated on LB plates for the cell titre and to calculate cfu/ml. MMC was added to the cultures to the final concentration of 0.5 μg/ml, the samples were withdrawn after 30 min and 60 min time intervals. The samples were then diluted and plated on appropriate LB plates. The cell titre (cfu/ml) value in each case was calculated based on the colony counts after 36 h incubation at 30 °C. The percentage survival of each of these strains at different time intervals after MMC treatment was calculated by taking the cfu/ml at 0 min sample (MMC untreated) as 100%. Survivors at 30 min and 60 min after