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Using Transposon Mutagenesis to Find an Alternative Resolvase in an *Escherichia coli* Cells Lacking RuvABC

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Abstract: This study was undertaken to identify an unknown resolvase in an *E. coli* strain lacking RuvABC (N4237) by using transposon mutagenesis. One out of 10000 clones was retained for further study as it was resistant to UV light and mitomycin C. The result of transductional mapping and PCR sequencing showed that Tn*10kan* inserted upstream of *rusA* gene and expression of this gene improved survival. Thus, results did not show the presence of new resolvase in *E. coli* cells.

Key words: Recombination, fork repair, resolvase, RuvABC, *rusA*

INTRODUCTION

Homologous recombination is a fundamental cellular process that promotes the repair of Double Strand Break (DSB) and the rescue of replication forks that have stalled at lesions (McGlynn and Lloyd, 2000, 2002; Michel *et al*., 2001).

In this process the RuvA, RuvB and RuvC proteins of *E. coli* encoded by *ruvA*, *ruvB* and *ruvC* genes, respectively are associated with processing of Holliday junction. RuvA and RuvB form a complex that promotes ATP-dependent branch migration of Holliday Junctions (HJ), a process that is important for the extension of heteroduplex DNA. The RuvC protein appears to interact with RuvAB-junction complex to form what has been referred as the RuvABC resolvasome that mediates junction cleavage (West, 1997).

In repair of stalled fork, it was suggested that rewinding of the parental strands and annealing of the nascent strands drive the fork back from the lesion and generate a structure resembling a HJ from a fork. The resolution of this structure by RuvABC provides double strand break that promotes recombination. Thus, RuvABC protein is required for initiation and termination of recombination in this fork repair pathway (McGlynn and Lloyd, 2000; Michel *et al*., 2001).

Strains lacking RuvA, B or C are unable to process junctions and are equally sensitive to DNA damaging agents such as UV light and mitomycin C (MC) (Mahdi *et al*., 1996). Due to the importance of these proteins, genes encoding of RuvA, RuvB and RuvC are present in many organisms. In microorganisms having RuvC, including *E. coli*, some have another resolvase, namely RusA. RusA is a HJ resolvase encoded by *rusA* gene of cryptic lambdoid prophage DLP12 of *E. coli* K-12. RusA is normally inactive and deletion mutations showed no obvious effect on recombination or sensitivity to UV light (Mahdi *et al*., 1996). However, it can be activated following the insertion of IS*2* or IS*10* containing promoter elements upstream of *rusA* to activate transcription (Mahdi *et al*., 1996). Meanwhile, in some microorganisms except *E. coli*, RusA is the only resolvase that has been already known, such as *Aquifex aeolicus* (Sharples *et al*., 1999).

Holliday junctions can also be processed in *E. coli* by RecG and RusA proteins in the absence of RuvABC. RecG is a DNA helicase that like RuvAB can drive branch migration of HJ and other branched structure (Lloyd and Sharples, 1993). It was shown that it can also form a HJ from a replication fork (McGlynn and Lloyd, 2000; Singleton *et al*., 2001). Then, RusA cleaves the point of crossover (Sharples *et al*., 1994; Chan *et al*., 1998).

Moreover, Mus81 is an endonuclease in yeast and mammals that like RuvC resolves Holliday junction (Osman and Whitby, 2007). The ability of RusA to suppress the UV and MMS sensitivities of a *mus81* mutant is consistent with the involvement of Mus81 in repair of stalled forks (Osman and Whitby, 2007).

On the other hand, there are some microorganisms that do not have either RuvC or RusA, including *Mycoplasma* sp. (Sharples *et al*., 1999). It suggests either they may have an unknown resolvase or they may use a repair pathway that is not dependent on recombination. Examples of these repair pathways have been presented by Trautinger *et al*. (2005) and Heller and Marians (2006).

The aim of this study was to find an unknown resolvase that promote survival in UV irradiated cells lacking RuvABC.

Table 1: *E. coli* strains used in this study

Strains	Relevant genotype	Relevant phenotype	Source or reference
MG1655	Wild type, prototroph	UV^R MCR	Lloyd and Sharples
N4237	*ruvAC65*	UV^{WR} MC^S	Lloyd and Sharples
N3598	*purE85 ruvA63*	UV^S MC^S	Lloyd and Sharples
RJ1046	*ruvAC65 orf96*	UV^R MC^R	This study
RJ1054	*orf96*	UV^R MC^R	This study

All strains are MG1655 derivatives. R, S and WR are abbreviation of resistance, sensitive and weak resistance, respectively

MATERIALS AND METHODS

Bacterial strain: This study was conducted couple of months ago. *Escherichia coli* K-12 strains used in this study are shown in Table 1. λNK1327 carries Tn*10kan plac* as described previously by Kleckner *et al.* (1991). P1*vir* is a virulent mutant of P1. IPTG (isopropyl-β-D-thiogalactopyranosid) was used in LB broth at 0.022 mg mL^{-1} to induce transcription from the *lac* promoter.

Media and general methods: LB broth and agar were used for bacterial culture. Media recipes and procedures for strain construction by P1*vir*-mediated transduction, testing sensitivity to MC and measuring survival of UV irradiated cells have been cited previously by Jaktaji and Lloyd (2003). UV survival values are means of three or four independent experiments.

Transposon mutagenesis: Tn*10kan* insertions were generated by infection of strain N4237 with λNK1327 and selection for kanamycin resistant clones at 42°C as described previously. Colonies of resistant clones were regrown in regular arrays and tested for sensitivity to MC and UV light using plate assays as described previously by Jaktaji and Lloyd (2003).

PCR amplication and DNA sequencing: Chromosomal DNA was extracted as cited previously by Jaktaji and Lloyd (2003). Location of insertion upstream of *rusA* gene was identified by sequencing PCR products amplified from chromosomal DNA using *rusA* specific primer 5′-TGGCGAAGAAGCTTTGCCATATTA-3′ and IS*10* specific primer 5′-CACCTATGTGTAGAACAGTATA-3′.

RESULTS

Strain N4237 was mutagenised using a Tn*10kan* element as described above. km^R clones showing evidence of increased growth on MC agar plates irradiated with UV were purified and further tested. Using this screen, some 10000 Km^R derivatives of N4237 were tested.

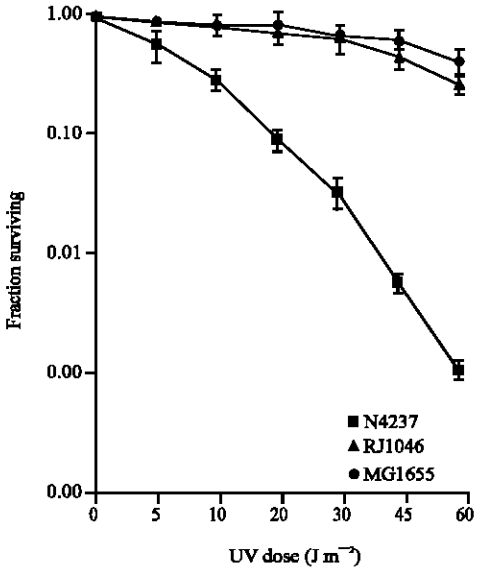

Fig. 1: The effect of insertion mutation on UV sensitivity

The RJ1046 clone was retained for study because it had increased resistance to both UV light (Fig. 1) and MC (data not shown) compared with the N4237 parent and MG1655 wild type strains. Since, the Tn*10kan* element used carries the *plac* promoter, it is possible that it activated the expression of a poorly transcribed gene that could promote repair (Kleckner *et al*., 1991).

It was shown that *purE* has 50% linkage to *rusA* (Mandal *et al.*, 1993). To see if RJ1046 carries an insertion that activates *rusA*, linkage of the Tn*10kan* insertion to *purE*85::Tn*10* in the *ruvA*63 strain N3598 was examined. P1 grown on RJ1046 was used to transduce strain N3598 to pur^+. These transductants (100) were screened for sensitivity to Km and Tc using plate assays. The inheritance of the $purE^+$ donor marker is associated with loss of Tn*10*. All pur^+ transductants proved MC^R. Also 47 out of 100 proved Km^R. These results indicated that the Tn*10kan* insertion in RJ1046 was linked to *rusA*. To confirm above results PCR amplification of the fusion and DNA sequencing were conducted as described above using strain RJ1054. The data drawn from PCR analysis showed an insertion is in *orf*96 upstream of *rusA* (Fig. 2).

As mentioned above the Tn*10kan* element carries the *plac* promoter. To see if induction of this promoter to over express *rusA* could further improve survival, the RJ1046 was tested for sensitivity to UV light in the presence and absence of IPTG (an inducer of *plac* promoter). The results showed that addition of IPTG had minor effect on UV survival of RJ1046 (Table 2). This indicates that the presence of the insertion is sufficient to allow enough expression of *rusA* to promote repair.

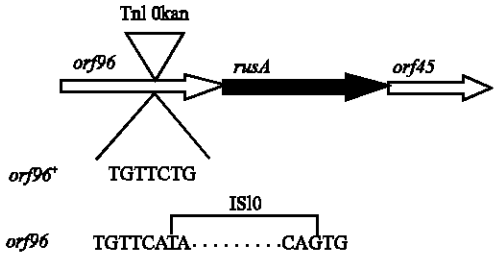

Fig. 2: Location of Tn*10kan* insertion in the *orf96* gene

Table 2: The effect of expression (IPTG minus) and over expression (IPTG plus) of *rusA* on UV survival

Strains	IPTG	Fraction (10 J m^{-2})	Surviving (30 J m^{-2})	UV dose (60 J m^{-2})
RJ1046	+	0.87	0.71	0.31
	-	0.85	0.66	0.27

The data are means of three independent experiments. Errors were less than 10% of the mean values

DISCUSSION

RuvC resolvase in conjunction with RuvAB branch migration motor produces RuvABC resolvasome (West, 1997). RuvABC involves in breaking the stalled fork and providing substrate for initiation of recombination. This protein also participates in processing of recombination intermediate to convert it to fork structure (McGlynn and Lloyd, 2000, 2002; Michel *et al*., 2001).

Homologs of RuvC protein have been found in many organisms from phages to human. Constantinou *et al*. (2001) found a combined branch migration and resolution activity in mammalian cell extracts. The human and yeast Mms4-Mus81 endonuclease (homolog of RuvC) arise when DNA replication is blocked by damage or by nucleotide depletion (Constantinou *et al*., 2001; Chen *et al*., 2001; Osman and Whitby, 2007).

In an attempt to find a substitute for RuvABC protein in *E. coli* cells lacking RuvABC, one UV and MC resistant clone, RJ1046 was obtained. By transductional mapping the location of Tn*10kan* was found which is near *rusA* gene. Moreover, by PCR sequencing the exact location of Tn*10kan* insertion was determined. It is in *orf96* gene, upstream of *rusA* gene that activates the expression of it. RusA is the Holliday junction-specific endonuclease (Sharples *et al*., 1994; Chan *et al*., 1998) that needs a branch migration protein, RecG to act (Lloyd and Sharples, 1993). However, unlike RuvC, it does not need to bind with a DNA branch migration protein like RecG or RuvAB to be activated (Rafferty *et al*., 2003).

This study and earlier study findings by Mahdi *et al*. (1996) that showed deletion or over expression of *rusA* in the presence of IPTG has no obvious effect on recombination and sensitivity to UV light suggests that although RusA can be activated in the absence of RuvABC, it is not vital for cell survival. Moreover, McGlynn and Lloyd (2000) found that activation of RusA in *ruv* mutant lacking *recB* has severe effect on sensitivity to UV light.

Finally, this study could not find an unknown resolvase in *E. coli* cells. This suggests that in the absence of RuvABC, *E. coli* cells may use an alternative non-recombinational repair pathway to rescue stalled fork. This might be a case for microorganisms that do not have both RuvC and RusA. A model of this non-recombinational repair pathway type was presented by Lloyd and Sharples (1993) and Trautinger *et al*. (2005). More recently, it was suggested that even in the presence of RuvABC, cells could have a strategy to prevent unnecessary recombination during replication fork repair (Mahdi *et al*., 2006).

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