Revista Latinoamericana de Microbiología

Volumen *Volume* **44** **Número** *Number* **2** **Abril-Junio** *April-June* **2002**

Artículo:

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Revista Latinoamericana de MICROBIOLOGÍA

ORIGINAL ARTICLE

Vol. 44, No. 2
April - June. 2002
pp. 58 - 64

Rat DNA polymerase beta substitutes the repairing activity of DNA polymerase I in the lethal effect of UV light

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ABSTRACT. The aim of this work was to search if the rat DNA polymerase beta can substitute the capability of DNA polymerase I to repair damage caused by the UV light in *Escherichia coli*. The *ori*C origin of replication from pβ5 was replaced by the *rep* origin from pSC101 and named pβ6. The presence of pol beta in the new construct was verified by PCR. *E. coli pol*A-1 (WP6) was transformed with pβ6. A protein with size similar to DNA Pol beta (40 kDa) was shown in the cell free extracts carrying pβ5. In WP6/pβ6 cell free extracts a slightly smaller protein was observed instead of the 40 kDa. DNA Pol beta was revealed by western analysis, with polyclonal antibodies, in strains with pβ5. Yet, it was not detected in the western from WP6/pβ6. A moderate change in UV resistance was observed in strains carrying pβ5. However, in *pol*A1 carrying pβ6 (WP6/pβ6), irradiated with 60-90 J/m^2 of UV light, the viability was increased by more than four orders of magnitude, when compared with the *pol*A1 (WP6) strain, reaching approximately the same UV resistance as the strains with DNA polymerase I. The results suggests that probably Pol beta is rapidly degraded in the cell free extracts from WP6/pβ6 and, it repairs the lethal effect of the UV light in *E. coli*.

Key words: DNA polymerase beta, *E. coli*, *oriC* origin replication.

RESUMEN. El objetivo de este trabajo fue investigar si la capacidad de la DNA polimerasa I de reparar el daño letal causado por la luz ultravioleta en *Escherichia coli* puede ser sustituida por la DNA polimerasa beta de rata. El origen de replicación *ori*C del plásmido pβ5 fue reemplazado por el origen de replicación *rep* de pSC101 y denominado pβ6. La presencia del gene pol β en el nuevo plásmido, se verificó por PCR. Con este plásmido (pβ6) se transformó a una cepa de *Escherichia coli pol*A-1 (WP6). Por medio de PAGE-SDS, se demostró que los extractos libres de células de las cepas portadoras del plásmido pβ5 producían una proteína de peso similar al de la DNA polimerasa beta de rata (40 kDa), y en la cepa portadora de pβ6 una proteína de peso molecular ligeramente inferior en lugar de la proteína original. El análisis "western", con anticuerpos policlonales, reveló que las cepas con pβ5 producían la DNA polimerasa beta. No obstante, no se detectó la enzima en el extracto de la cepa con pβ6. Las cepas con pβ5 mostraron un cambio moderado en la resistencia a la luz ultravioleta respecto a las cepas control. En cambio, la cepa *pol*A-1 con pβ6 recuperó, a dosis de 60-90 J/m^2, más de cuatro logaritmos de viabilidad comparado con la cepa polA-1, alcanzando aproximadamente los mismos niveles de resistencia que las cepas portadoras de la DNA polimerasa I. Los resultados sugieren que la DNA polimerasa de rata, aunque probablemente se degrada rápidamente en los extractos de la cepa recombinante WP6/pβ6, es capaz de reparar en *Escherichia coli* el daño letal causado por la luz ultravioleta.

Palabras clave: DNA polimerasa beta, *E. coli*, origen de replicación *ori*C.

INTRODUCTION

Mammalian DNA polymerase beta is the smallest known eukaryotic polymerase. Its main role is to repair DNA damage caused by methylating agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and methyl methane sulfonate and by the cross linking antineoplastic drugs mitomycin C and mafosfamide (Idriss, et al, 2002; Sobol et al, 1996; Ochs et al, 1999). The correction of damage by this enzyme, by base excision repair, is done with high fidelity (Osheroff et al 1999a) and, the mechanisms involved in this accurate repair are currently under careful study (Beard and Wilson, 2000; Beard et al, 2002; Yang et al, 2002). In its absence cells are induced to apoptosis and chromosomal breakage. It is able to synthesize DNA in short DNA gaps during DNA repair. It is composed of two specialized domains that contribute essential activities to base excision repair (BER). Its amino-terminal 8kDa domain possesses a lyase activity necessary to remove the 5'-deoxyribose phosphate (dRP) intermediate generated during BER. The larger (31 kDa) domain has nucleotidyl transferase activity (Osheroff et al, 1999b). The failure to remove the dRP group may initiate alternate BER pathways (Beard and Wilson, 2000). The role of DNA polymerase beta in BER has been established also by reconstitution from purified proteins (Nicholl et al, 1997). The participation of DNA polymerase beta in a "long patch" BER pathway has also been studied by reconstitution from purified proteins (Klungland and Lindahl, 1997). It also was shown that, in excess, error-prone Pol beta participate in nucleotide excision repair (NER) (Canitrot et al, 2000). In 1992 Sweasy and Loeb found that mammalian DNA

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polymerase beta substitute for DNA polymerase I during DNA replication in *Escherichia coli*. They also found that rat DNA polymerase beta replace both the replicative and repair (BER) functions of DNA polymerase I in *Escherichia coli* (Sweasy and Loeb, 1992; Sweasy and Loeb, 1993). The repair by DNA polymerase beta mutator can produce mutations as was shown with mutant Pol beta-14 which increases the spontaneous mutation rate in *Saccharomyces cerevisiae* (Clairmont and Sweasy, 1998) and with Pol beta D276E which exhibited a 25-fold increase both in the catalytic and misincorporation efficiencies (Skandalis and Loeb, 2001). Dominant negative rat DNA polymerase beta mutants did not confer sensitivity to UV light (Clairmont and Sweasy, 1996). However, it has been observed that DNA polymerase beta can repair DNA damage, by nucleotide excision repair (NER), caused by UV light in eukaryotic cells (Canitrot et al, 2000). The aim of this work was to search if DNA polymerase I can be substituted by DNA polymerase beta in the repair of DNA damage caused by UV light.

MATHERIAL AND METHODS

Table 1. *E. coli* strains and plasmids used. WP1 and WP6 are isogenic (Witkin, 1975), except that WP6 is a polA-1 strain. uvrA66 is an *E. coli* strain used as reference by its known UV sensitivity (Kiyosawa et al, 2001). pβ5 has an *ori*C as origin of replication while pβ6 has *rep* as its origin of replication.

Strain	Genotype	Source
JM109	recA1, supE44, endA1, hsdR17, gyrA96 thi-1, Δ(lac-pro AB), F'[traD36, proAB, lac^q, lacZΔM15].	K. L. Beattie.
JM109/ pUC118	recA1, supE44, endA1, hsdR17, gyrA96 thi-1, Δ(lac-pro AB), F'[traD36, proAB, lac^q, lacZΔM15]/pUC118.	This work
JM109/ pβ5	recA1, supE44, endA1, hsdR17, gyrA96 thi-1, Δ(lac-pro AB), F'[traD36, proAB, lac^q, lacZΔM15]/pUC118/pβ5.	K. L. Beattie.
WP1	lon1, SulA1, $malB^+$, trpE65.	K. L. Beattie.
WP1/pβ5	lon1, SulA1,$malB^+$, trpE65, amp^r/pUC118/Pβ5.	Hernández*
WP6	lon1, SulA1, $malB^+$, trpE65, polA-1.	K. L. Beattie.
WP6/pβ6	lon1, SulA1, $malB^+$, trpE65, polA-1/pβ6	Hernández*
uvrA66 Beattie	lon1, SulA1, $malB^+$, trpE65, uvrA6.	K. L.

The DNA polymerase beta polyclonal antibodies were a generous gift of SH Wilson. The Ab anti-polyclonal antibodies conjugated to horse radish peroxidase and the oligonucleotides were also a generous gift of Dr. Quintana-Hau.

The restriction enzymes and DNA molecular weight markers were acquired from New England Biolabs. The protein molecular weight markers were from Sigma Chemical Co. When required the solutions were sterilized by filtration in Millipore with membranes having 0.22 μm pores.

Construction of pβ6: Plasmid pβ6 was constructed as follows. Plasmidic DNA from p 5 was obtained from E. coli JM109/pβ5. Digested with the restriction enzymes AlwN1 and AflIII to eliminate the oriC origin. The ends filled by DNA synthesis with the Klenow fragment of the DNA polymerase I. The *rep* origin of replication was obtained by excision of pSC101 (by cutting with the restriction enzyme *Hinc*III, followed by separation by electrophoresis in 1% agarose gel, and elution of the appropriated DNA band. The DNA fragment with the *rep* origin and the digested pβ5 without *ori*C were ligated and transfected in *E. coli* DH5a. The construction was verified by its mobility in 1% agarose gel, by restriction analysis and, PCR recovery of the DNA fragment containing DNA polymerase beta. Finally, the plasmidic DNA was isolated and used to transfect *E. coli* WP6.

Plasmid DNA minipreps: were prepared from 3 ml cultures by the alkaline lysis method according to Sambrook, 1989.

PCR: Plasmidic DNA was submitted to amplification by PCR with oligonucleotides 676 (5´-CCGGATCTGCAAATGAGCAAACGCAAGGCCCCG-3´) and 677 (5´-CAGCCCGGGTCATTCGCTCCGGTCGTTGGGTTC-3') to generate a 1029 bp DNA fragment containing the DNA polymerase beta gene (Zmudka et al, 1986). The mixture of reaction contained: Plasmidic DNA (0.03 mg/ml) 1 ml, primers (25 pmols/ml) 1 ml each, 10 mM dNTPs 2, 10X PCR buffer 10 ml, 30 mM $MgCl_2$ 5 μl, HPLC water 74 μl and *Taq* DNA polymerase 2.5 U. The incubation was done in a MJR minicycler at 95ºC 5 min, followed by 30 cycles at 94ºC 1 min, 60ºC 1 min, 75ºC 1 min and, 75ºC 5 min for the final extension.

Electrophoresis of DNA: 10 ml of PCR product samples were submitted to agarose gel electrophoresis following the instructions described by Sambrook, 1989. Then stained with ethidium bromide and revealed with UV light.

Induction of DNA polymerase beta: Pairs of tubes containing 3 ml of YT media (Sambrook, 1989) with or

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without ampicilline (50 µg/ml) were inoculated with 30 µl of overnight cultures, incubated 3 h at 37ºC. Then one of the cultures was induced with 1 mM IPTG. Both tubes were incubated additionally 6 h more. Aliquots of 1.5 ml were centrifuged 30 seconds at 12,000 g. The pellet was resuspended in 1 ml of sterile 0.15 M sodium chloride and centrifuged in the previous conditions. The cells were resuspended in 50 µl of HPLC water. Added with 100 µl of lysis solution (2.5 ml tris-HCl 0.5 M pH 6.8, 4 ml 10% SDS, 0.5 ml distiled H_2O and 2 ml glycerol were mixed and stored at –20ºC in aliquots of 900 µl. Just previous to its use 100 µl of mercaptoethanol and 10 µl of loading buffer were added). Boiled by 15 min. Cooled on ice and. Stored at –20ºC.

The samples were submitted to PAGE-SDS and revealed with Coomasie blue R-250 (0.125 mg/ml).

Determination of sensitivity to UV: Growth curves were determined for all the strains. The following procedure was done with each strain. A 3 ml culture was grown overnight in YT media at 37ºC. Pairs of 125 µl Erlenmeyer flasks containing 25 ml of YT media were inoculated with 125 ml of the previous culture and incubated at 37ºC until reaching the middle of the logarithmic phase of growth. IPTG (1 mM) was added to one of the flasks and then, both flasks were incubated 4 h more. The cells were pelleted by centrifugation at 3,000 g. The pellet resuspended in 5 ml of 0.15 M NaCl. Suspensions of 1 x 10^9 UFC/ml were used for the UV sensitivity test.

The cell suspensions were placed in a Petri dish, submitted to a circular movement (200 rpm) in a Thomas shaker and, irradiated with a germicidal UV lamp. The UV doses were determined with a UVX digital radiometer having an UVX-31 sensor. Aliquots of 10 ml were taken at several UV doses, the viability of the cells (UFC/ml) and the curves of UV sensitivity determined for each sample. Each experiment was done by triplicate and the points of the curves are the media of the data. All the dilutions and incubations were done in the dark.

Western blott: A 3 ml culture was grown overnight in YT media at 37ºC. Tubes with 3 ml of YT media were inoculated with 10 µl of the overnight and incubated at 37ºC until reaching the logarithmic phase of growth. IPTG (1 mM) was added to one of the flasks and then, both tubes were incubated further 4 h more. Aliquots of 1.5 ml were centrifuged 30 seconds at 12,000 g. The pellet was resuspended in 1 ml of sterile sodium chloride physiological solution and centrifuged in the same conditions. The cells were resuspended in 50 µl of HPLC water. Added with 100 µl of lysis solution and homogeneized. Boiled by 15 min, cooled on ice and stored at –20ºC until used for electrophoresis. The samples were submitted to PAGE-SDS and then transferred to nitrocellulose membranes in an immunotransference chamber (2 h at 100 V). The gel was revealed with Ponceau S solution (10X = 2 g Ponceau S, 30 g trichloroacetic acid, 30 g sulfosalicilic and water to 100 ml) and the membrane washed until getting perfect definition of the bands. Then strips of the membrane having the protein bands were cutted and placed in a multichannel tray. Each strip was treated as follows: 2 ml of PBS were added, pour off, wash with water 30 min in 1 ml regeneration buffer (1% Triton-X100 in PBS), pour off, wash with water, then 1 ml of blocking solution (2% milk powder) was added, incubate 60 min with gentile shaking, eliminate the blocking solution, add 1 ml of the primary antibody at the convenient dilution, incubate 4 h at room temperature with slow shaking, eliminate the antibodies, wash 4 times, 2 minutes each, with washing solution I (0.01% Tween 20 in PBS) and, another four times in washing solution II (PBS = 6.184g boric acid, 9.356g $Na_2B_4O_7.H_2O$, 4.384g NaCl per liter), incubated in 1 ml (diluted 1:3,000) of the secondary Ab conjugated to horse radish peroxidase, eliminate the antibodies, incubate with the substrate until revealing the protein reacting bands, eliminate the reagents, wash with water, dry and keep in the dark until recording.

RESULTS

The presence of the pol β gene in the plasmids was assessed by observing the electrophoretic mobility, by restriction analysis and by the observation of the PCR product (1029 bp). As expected the 1029 bp band PCR product was obtained from JM109/pβ5 WP1/pβ5 and from WP6/pβ6 (Fig. 1) but not from JM109 (whose genotype is given as part of JM109/pUC118), WP1 or WP6.

The expression of the DNA polymerase beta gene was followed first by the formation of a 40 kDa protein in the cell crude extracts from bacterial hosts transporting plasmids with pol beta this was done in JM109/pβ5 and WP1/pβ5 (Fig. 2), however, in JM109/pβ5 the protein was inducible while in WP1/pβ5 it was constitutive. In the case of WP6/pβ6 the induced protein showed a slightly faster mobility and was also no inducible (Fig 2). Another criteria to follow the expression of the gene was the detection of the corresponding protein by western analysis. A band with the expected mobility was seen in the extracts from JM109/pβ5 and WP1/pβ5 strains. No band was observed in the extract from WP6/pβ6 (Fig. 2B).

The sensitivity to UV light is shown in Figures 3, 4 and 5. Panel A from Figure 3 shows the survival curve of JM109/pUC118 and JM109/pβ5. The recombinant bacteria having pol beta (JM109/pβ5) was 1 (at 45 J/m^2) to 2 logarithms (at 90 J/m^2) more resistant than the bacteria with the

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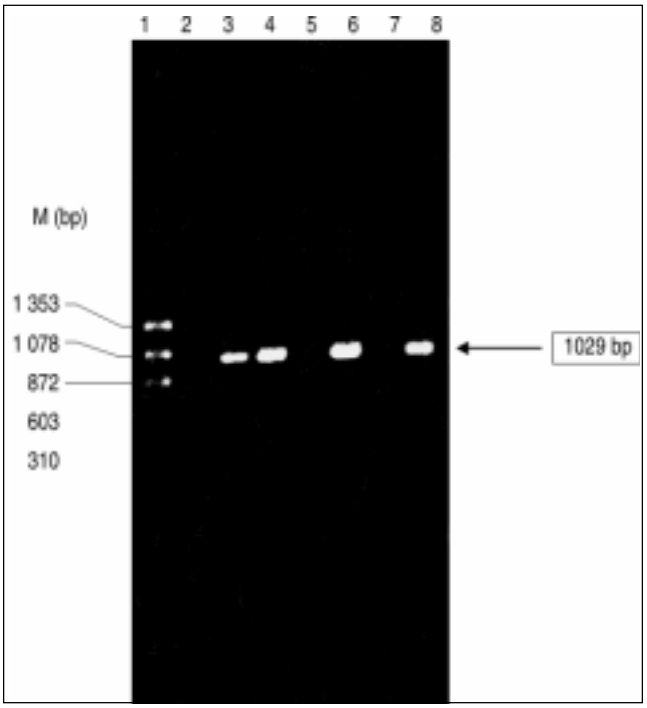

Figure 1. *Detection of the DNA polymerase beta gene in the strains by PCR. Lane 1 is the DNA ladder (ΦX174-*Hae*III). Lane 2 negative control without DNA. Lanes 3 and 4 corresponds to pUC118/pβ5 samples. Lane 5 corresponds to the control sample from WP1. Lane 6 corresponds to the sample from WP1/pβ5. Lane 7 corresponds to the control sample from WP6. Lane 8 corresponds to the sample from WP6/pβ6. The arrow points to the position of the PCR product (1029 bp).*

vector lacking pol beta. The opposite behavior was observed for WP1/pb5 (more sensitive) in comparison with WP1 (more resistant). In the case of WP6, this strain was much more sensitive (0.01% of survival at 30 J/m^2 and, 0.0001% of survival at 90 J/m^2). However, when the same strain was transporting the DNA polymerase beta (WP6/pb6) it recovered a UV resistance similar to the wild type *E. coli* strain (JM109).

In Figure 4 are shown the survival curves for the WP6 and uvrA6 *E. coli* strains. Both strains had similar UV resistance.

The resistance of JM109/pβ5 to UV was also determined at increased times of incubation after adding IPTG. In Figure 5 is shown that the sensitivity of the strain increases with the time of culture after adding IPTG.

DISCUSSION

As it has been previously shown plasmids with an *ori*C origin of replication are unable to grow in *E. coli* polA strains (Filutowicz and Roll, 1990). Therefore, the *ori*C origin of replication present in pβ5 was substituted by the *rep* origin of replication from pSC101. The new plasmid was named pβ6.

Another critical control for this study was the use of isogenic host strains, having the *polA-1* mutation as the only difference. For this reason were selected the *E. coli* strains WP1 and WP6 which, respectively, contains or lacks DNA polymerase I (Witkin, 1975).

The presence of the insert in the plasmid was determined by PCR. The same 1029 bp PCR product reported by Date et al, 1988, was obtained from JM109/pβ5, WP1/pβ5 and WP6/pβ6 (Fig. 1), while it was absent in the control strains (JM109/pUC118, WP1 and WP6). This data suggest that the band corresponds to the DNA polymerase beta insert and, that the same insert is present in pβ5 and pβ6.

It is important to associate the production of the enzyme with its biological effect. The determination of Pol beta production was first done by the identification of the band of protein of the appropriated size by PAGE-SDS analysis. Cell crude extracts from induced and non induced cultures were analyzed. The original strain (JM109/pβ5), as previously reported (Date *et al*, 1988), formed the expected 40 kDa inducible protein (Fig. 2). The 40 kDa protein was also produced by WP1/pβ5. However the production of this protein in WP1/pβ5 was constitutive. Therefore, the change in this strain causing the constitutive synthesis of the enzyme at high levels is possibly due to differences in the genetic pool of both *E. coli* strains. In the case of WP6/pβ6 an intense and constitutive protein with slightly faster electrophoretic mobility was seen, instead of the expected protein of 40 kDa. We do not know the source of the change in the mobility. But, it can be a shorter form of the protein caused by some unknown problem in the construction of pβ6 or by changes in transcription or translation of the gene.

Another better form to identify the enzyme is to reveal its presence by western analysis. Antibodies for DNA polymerase beta were provided by SH Wilson and used to search the formation of DNA polymerase beta by the strains. The results (Fig. 2) confirmed the production of the enzyme by the recombinant strains JM109/pβ5 and WP1/pβ5. In JM109/pβ5 was produced in the presence of IPTG while in WP1/pβ5 DNA polymerase beta was produced both in the presence and absence of inducer. Yet, no signal of enzyme was seen in WP6/pβ6. Therefore, the protein with bigger mobility detected in the previous assay, from WP6/pβ6 cell free extracts, was unable to react with the antibodies used.

To quantitate the resistance to UV light in the strains, growth curves were determined with the purpose to establish the period of logarithmic phase in each strain, and to search the effect of IPTG on the growth. No significative differences were seen, with or without inducer, between the strains, except for WP6/pβ6, which had a longer lag phase in both conditions.

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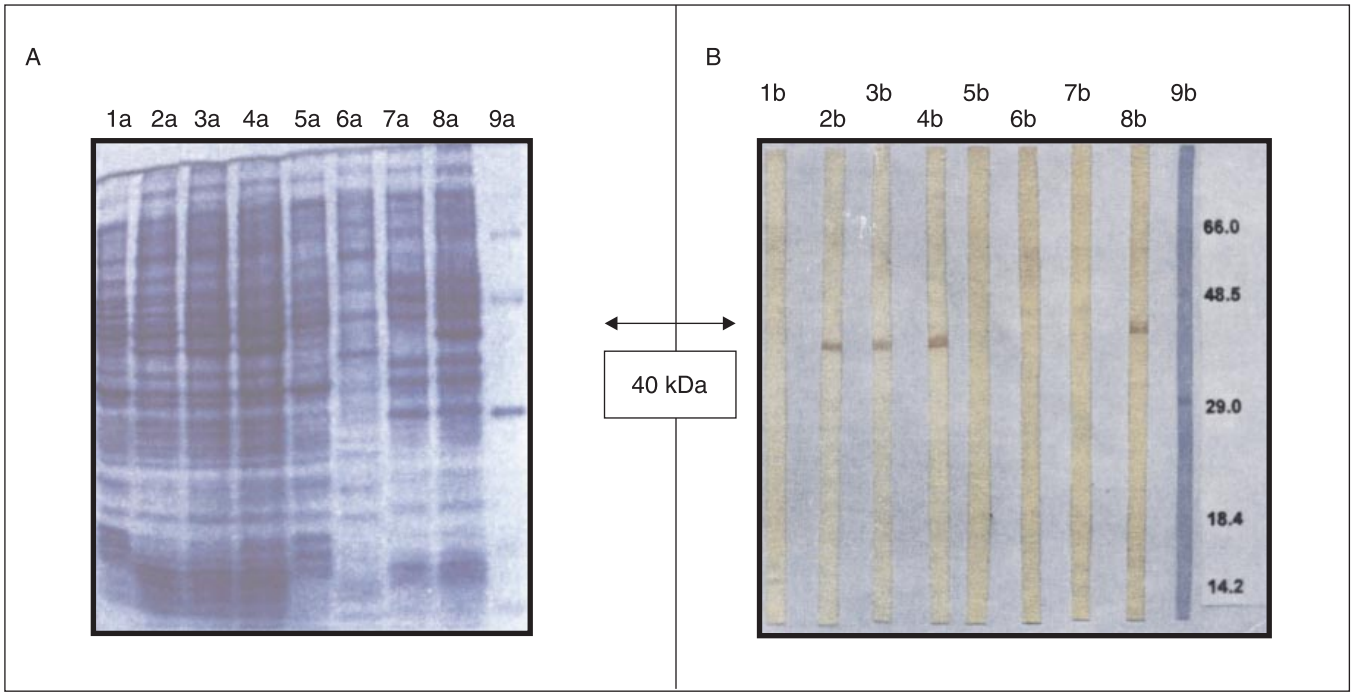

Figure 2. *Kinetics, and controls, of DNA Pol beta expression in WP1/pβ5. Panel A: Electrophoretic analysis in PAGE-SDS at several times of induction with IPTG. Lane 1a is a control (WP1); lanes 2a, 3a y 4a are samples from WP1/pβ5 induced by 0, 2 and 4 h, respectively. All the other samples were obtained from cultures induced by 4 h. Lane 5a corresponds to WP6. Lane 6a corresponds to WP6/pβ6; lane 7a corresponds to JM109/pUC118. Lane 8a corresponds to JM109/pβ5 and lane 9a corresponds to the molecular weight marker given in kDa. Panel B: Immunodetection of Pol β. Lanes 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b and 9b correspond to the same samples from panel A.*

From the assays done with UV irradiation it was observed that the UV response was not affected by IPTG, except for JM109/pβ5, which has DNA polymerase beta under the control of the inducer. It was founded that, in general, the basal levels of DNA polymerase beta are increasing the UV resistance. As suggested by the bigger UV survival in JM109/pβ5 and WP6/pβ6, when compared with the control strains (JM109/pUC118 and WP6, respectively) lacking DNA polymerase beta. The most important protecting effect was seen in WP6/pβ6, which reached resistance levels resembling those in *E. coli* WP1 and JM109. The absence of DNA polymerase I in WP6 gave a strong correlation with its very low UV survival curve. The high UV sensitivity of WP6 was very similar to that observed in *E. coli uvrA6* (Fig. 4), which lacks one of the proteins involved in the UV excinuclease repair system (Kiyosawa, 2001). Similar UV sensitivity levels are seen when other genes of the UV excinuclease system are affected (uvrB, uvrC, recA, pol) (Bridges, 1976).

The UV sensitivity of WP1/pβ5 was bigger than WP1 when assayed 4 h after the addition of IPTG. However, WP1/pβ5 was more resistant than WP1 to UV light when tested at the middle of the log phase. By this reason the effect of the time (0, 1, 2, 3, and 4 h) of incubation, after the addition of IPTG, on the UV sensitivity. Figure 5 shows that the lethal effect of the UV light increases with the time of incubation (until four hours) in the presence of UV. The curve of growth of WP1/pβ5 (data not shown) indicates that after four hours of IPTG addition WP1/pβ5 is in the stationary phase of growth. Also the amount of rat DNA polymerase beta increases during this period of time. These data suggest that maybe the expression of some genes induced during the stationary phase of growth, such as *fis* and *Rpo*S, which are able to activate or repress the activity of many other genes (Xu and Johnson, 1995), are causing interference in the activity of the rat DNA polymerase beta. Another interesting possibility is, the inhibition of activity of the enzyme by fragments of the degradation of the enzyme that are still able to bind the substrate but have lost their its enzymatic activity. This work and others have shown that the enzyme (rat DNA polymerase beta) disappears from the cell free extracts in a short period of time. Also, Bhattacharyya and Banerjee, 1997, are demonstrated that the 14 and 16 kDa protein fragments arising from the degradation of DNA Pol beta keep their gapped DNA binding capacity (Husain *et al*, 1995) while they loose their polymerizing activity and are negatively acting on the DNA Pol beta activity.

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These fragments are unable to inhibit the Pol α and Pol I activities (Hussain et al, 1995). Therefore, if this is the mechanism of inhibition, the inhibitory effect is due to competition on the rat DNA Pol beta activity. However, it is also possible

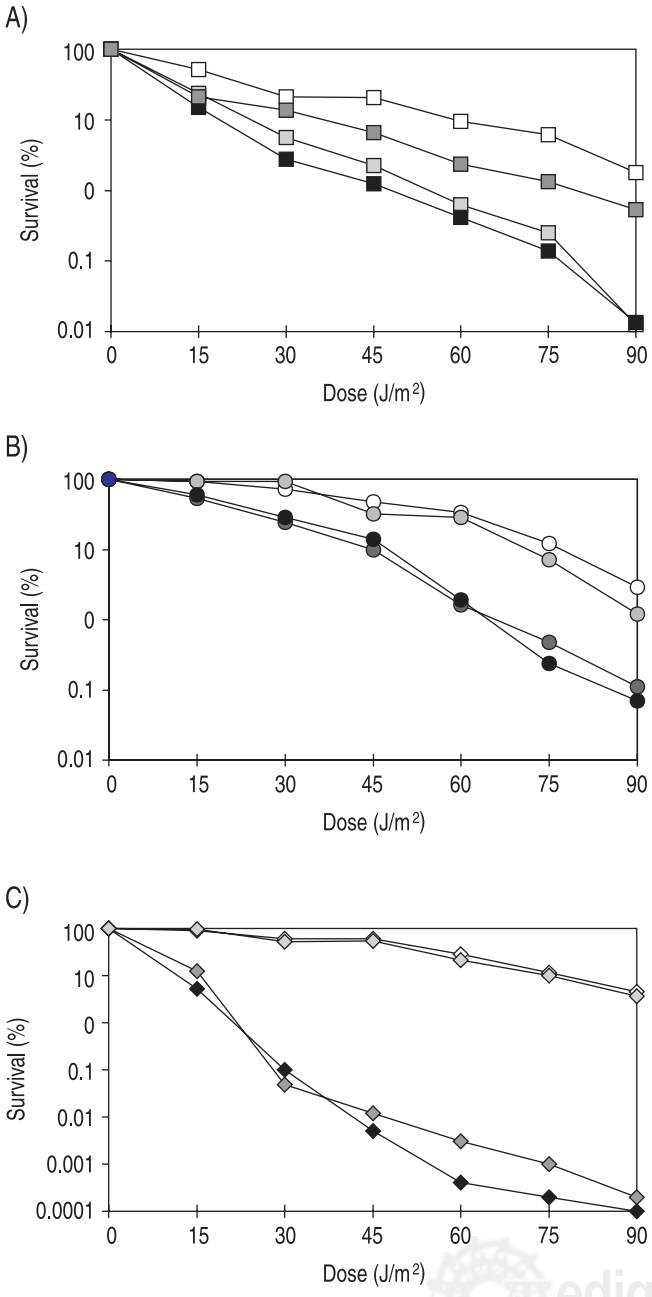

Figure 3. *UV sensitivity in the wild type and recombinant* E. coli *strains under induced and non induced conditions. Panel A) corresponds to JM109/pUC118 (□ and ■) and JM109/pb5 (□ and ■), panel B) to WP1(○ and ●) and WP1/pb5 (○ and ●) and panel C) to WP6 (◇ and ◆) and WP6/pb6 (◇ and ◆). Empty symbols correspond to growth in absence of IPTG. Filled symbols correspond to growth in presence of IPTG.*

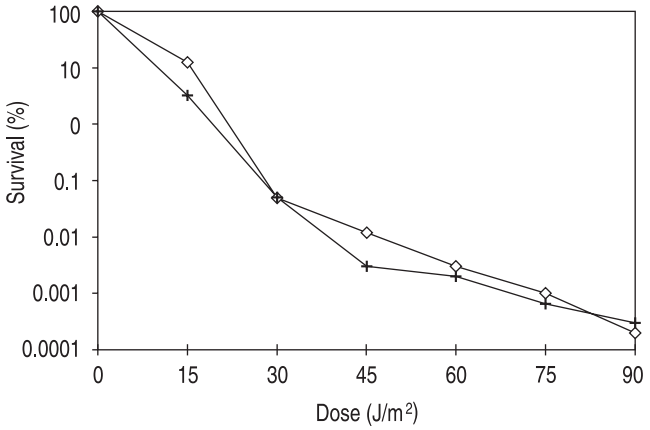

Figure 4. *UV sensitivity in WP6 and uvrA6* E. coli *strains. WP6 (◆) in presence of IPTG. uvrA6 (+).*

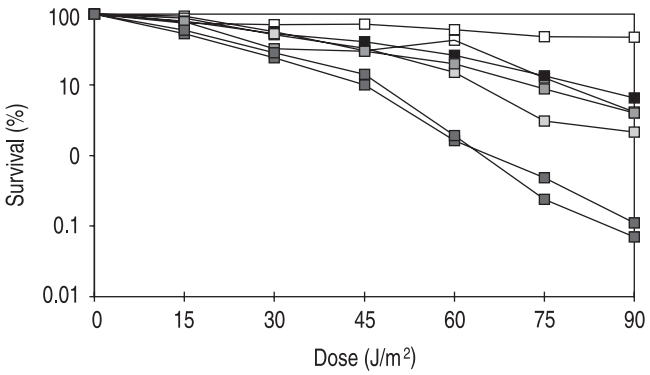

Figure 5. *Kinetics of UV survival at several induction times in* E. coli *WP1/pβ5. WP1/pβ5 at: 0.0 hrs (□), 1 hrs (□ and ■), 2 hrs (□ and ■), and 4 hrs (□ and ■) of induction. Empty and empty symbols are, respectively, in absence or presence of IPTG.*

that the high levels of Pol beta can compete with Pol I. Figure 5 shows that the sensitivity to UV light is bigger in WP1/pβ5 than in WP1 at the higher times of IPTG induction. Therefore, the hypothesis of the competence between the enzymes can be a plausible explanation for the results obtained.

The immunoassay to detect DNA Pol beta was done also after 4 h of induction. The DNA Pol beta, with the expected size, was detected in the extracts from JM109/pβ5 and WP1/pβ5 but not in the negative controls (JM109/pUC118, WP1 and WP6) (Fig. 5). However, the extract from WP6/pβ6 was also negative. We do not have an explanation for this result. Yet, a strong band of protein with slightly faster mobility, instead of the expected 40 kDa band, was seen in this strain, which suggests that it has a smaller size and that a faster degradation of DNA Pol beta occurs in this microorganism. Alternatively, the construction (pβ6) has some

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change that affects its size and immunological properties. Further experiments needs to be done to search these possibilities. The previous observation, together with the extraordinary high recovery in UV resistance in this strain when compared with its control (WP6), strongly suggest that the rat DNA polymerase beta is repairing the DNA damage caused by irradiation with UV light and, that rat DNA polymerase beta is replacing the repairing activity of DNA polymerase I in *E. coli*. Which agrees with the previous report of repair of DNA damage, caused for alkylating agents, in *E. coli* (Sweasy and Loeb, (1993).

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