ORIGINAL ARTICLE JGEB Vol. 7, No. 2, 2009 ISSN:1687-157X

Biochemical Characterization of *Arabidopsis thaliana* DNA Polymerase λ: Role in the Oxidative DNA Damage Bypass

Alessandra Amoroso[1], Lorenzo Concia[2], Paolo Longoni[2], Emmanuele Crespan[1], Rino Cella[2] and Giovanni Maga[1]

[1]Institute of Molecular Genetics, IGM-CNR, via Abbiategrasso, [2]Department of Genetics and Microbiology, University of Pavia, Via Ferrata, Pavia (Italy)

ABSTRACT

As obliged phototropic organisms, plants are continuously exposed to high levels of reactive oxygen species (ROS), generated within the cell as a result of the exposure to biotic stresses, sunlight and metabolic activity. The most frequent DNA oxidation damage generated by ROS is the 7,8-dihydro-8-oxo-guanine (8-oxo-G) adduct. When present in the replicating DNA strand, this lesion is highly mutagenic, leading to the insertion by the replicative DNA polymerases (pols) of an adenine in place of the correct cytosine. Here we present the first characterization of an error-free mechanism for 8-oxo-G bypass operating in crude extracts of *Arabidopsis thaliana*. For the first time, our results show that, similarly to what observed in mammalian cells, plant cells possess an efficient pathway for faithful translesion synthesis of oxidative DNA lesions.

Key Words: Arabidopsis, oxidative stress, DNA polymerase λ (Lambda), translesion synthesis.

Corresponding Author: Giovanni Maga
E-mail: maga@igm.cnr.it

Journal of Genetic Engineering and Biotechnology, 2009, 7(2): 11-16

INTRODUCTION

Despite having relatively stable genomes, all living organisms are subjected to damage by physical and chemical environmental agents (UV and ionizing radiations, chemical mutagens, fungal and bacterial toxins, etc.) and by free radicals or alkylating agents endogenously generated by metabolism *(Britt, 1999)*. DNA can also be damaged because of errors during its replication. The DNA lesions produced by these damaging agents could result in base change, base loss, base mismatch, base deletion or insertion, linked pyrimidines, strand breaks, intra- and inter-strand cross-links *(Bray and West, 2005)*. These DNA lesions can be either genotoxic or cytotoxic. Plants are most affected by the UV-B radiation of sunlight, which penetrates cells and damages their genome by inducing DNA-protein and DNA-DNA cross-links and oxidative damage through the generation of reactive oxygen species (ROS) *(Collins, 1999)*. ROS are produced not only through the action of exogenous agents, but also during normal cell metabolism. When ROS react with DNA, the most frequently generated lesion (10^3-10^4 per cell/per day) is 7,8-dihydr o-8-oxo guanine (8-oxo-G), which is potentially mutagenic *(Kamiya, 2003 and 2004)*. In fact, the presence of 8-oxo-G in the replicating strand can lead to frequent misincorporation of A opposite the lesion by the replicative DNA polymerases (DNA pols) α, δ and ε, leading to an error prone synthesis *(Amoroso et al., 2008)*. The subsequent error-free bypass of the lesion requires a specialised DNA polymerase that can catalyse the correct incorporation of C opposite 8-oxo-G during the re-synthesis step reconstituting a C:8-oxo-G base pair that could subsequently be repaired by the base excision repair (BER) mechanism *(Macpherson et al., 2005)*.

We have recently shown that the mammalian BER enzyme DNA pol λ, which belongs to the DNA pol family X, is very efficient in performing error-free translesion synthesis past the two major oxidative lesions 8-oxo-G and 2-hydroxy-adenine (2-OH-A) *(Krahn et al., 2003)*. Moreover, its fidelity and efficiency is enhanced two orders of magnitude by the human auxiliary proteins PCNA and RP-A. The mechanisms of DNA repair are best understood in micro-organisms and mammals, but the field has recently expanded to include both plants and lower animals. These recent advances in our understanding of the molecular DNA repair in higher plants include such aspects as the repair of UV-induced pyrimidine dimers, the correction of mismatched bases and the rejoining of double strand breaks. Less is known about the ability of plant DNA pols to synthesize opposite oxidative DNA damages.

In plants, the general knowledge about the DNA pol λ structure and functions is still limited *(Crespan et al., 2007)*. This study focused on the molecular mechanisms underlying the processes by which plant polymerases carry out translesion synthesis (TLS) during the nuclear replication to bypass the oxidative 8-oxo-G damage using *Arabidopsis thaliana* as a model organism *(Babiychuk et al., 1998)*.

We initially evaluated the overall polymerase activity present in *Arabidopsis* suspension cell extracts identifying the presence of DNA pol λ and analyzed the variation of polymerase catalytic activity in the presence of 8-oxo-G DNA damage. Depending on the efficiency of dNTPs incorporation opposite to the 8-oxo-G damage,

we sought to assess whether the mechanisms bypassing the oxidative lesions were either error-free or error-prone.

Our results show, for the first time, that an error-free bypass mechanism of 8-oxo-G is operating in *Arabidopsis thaliana.*

MATERIALS AND METHODS

Chemicals:
Deoxynucleotides were purchased from Gene Spin (Milan, Italy). All the other reagents were of analytical grade and purchased from Fluka or Merck. The 39mer, 32mer and 72 mer oligonucleotides either unlabelled or 5'-biotinylated, were purchased from MWG (Florence, Italy)

Oligonucleotides substrate:
All oligonucleotides were purified from polyacrylamide denaturing gels. Annealing of the 72mer, either undamaged or containing the 8-oxo-G lesion (8-oxo-dG-CE Phosphoramide, from Glen Research), with the 5'-labelled 39mer primer and the 32mer terminator oligonucleotides generated the 1nt gapped templates.

All oligonucleotides were purified from polyacrylamide denaturing gels.

The sequences are:
72 mer template
3'ATG TTG GTT CTC GTA TGC TGC CGG TCA CGG CTT AAG TGT **G**GC **G**GC CGC GGG TTG GAG **G**GC TTA TAG ATT ATG- 5'

39 mer primer
5'-TAC AAC CAA GAG CAT ACG ACG GCC AGT GCC GAA TTC ACA- 3'

32 mer primer
5'- CGC CGG CGC CCA ACC TCC CGA ATA TCT AAT AC- 3'

The 72 mer, either undamaged or containing the 8-oxo-G lesion (8-oxo-dG-CE Phosphoramidite, from Glen Research) and the corresponding primer were chemically synthesized and purified on a 12% (w/v) polyacrylamide, 7 M urea, 10% formamide gel. After elution and ethanol precipitation, their concentrations were determined spectrophotometrically. The bold letter in the sequence corresponds to the position of the 8-oxo-G lesion.

The 39 mer primer was labeled with Blue FAM Fluorescence group by the Eurofin Company. Each labeled primer was mixed with the complementary template oligonucleotide at 1:1:1 (M/M) ratio in the presence of 25 mM TrisHCl pH 8 and 50 mM KCl, heated at 75°C for 10 min and then slowly cooled down at room temperature.

***In vitro* polymerase assays:**
The reaction mixtures contained CE A and C in a 10 µl final volume constituted by: TDB Buffer, 0.01 mM (^{3}H)TTP) (1490 cpm/mol), dNTPs (1.5 Ci/mmol), 10 mM $MgCl_2$ and 0.5 mg poly(dA)/oligo(dT) 5:1 in presence of different concentrations of PCNA (0-100 ng). After incubation at 37°C for indicated time, 10 µl from each reaction tube were spiked on glass fiber filters GF/C and immediately, immersed in 5% ice-cold trichloroacetic acid (TCA) (AppliChem GmbH, Darmstadt). Filters were washed three times with 5% TCA and once with ethanol for 5 minutes, then dried and finally, adding EcoLume ® Scintillation cocktail (ICN, Research Products Division, Costa Mesa, CA USA), to detect the acid-precipitable radioactivity by PerkinElmer ® Trilux MicroBeta 1450 Counter.

***In vitro* translesion assays:**
For denaturing gel analysis of the DNA synthesis products, the reaction mixtures contained: dsDNA substrate (8-oxo-G damaged and control), TDB Buffer (50 mM Tris–HCl pH 7.5, 0.20 mg/ml BSA, 1 mM DTT, 2% Glicerole), 1 mM Mg^{++}, 0.01mM (^{3}H)TTP (1490 cpm/mol), different concentrations of dNTPs, A and C Crude Extracts. Human PCNA was used for the complementation of assays. Reaction mixtures were incubated for 25 min at 37°C and then stopped by addition of standard denaturing gel loading buffer (95% formamide, 10 mM EDTA, xylene cyanol and bromophenol blue), heated at 95°C for 5 min and loaded on a 7 M urea 12% polyacrylamide gel. The reaction products were analyzed by using Molecular Dynamics PhosphorImager (Typhoo Trio GE Healthcare) and quantified by Image Quant and GraphPad Prism programs.

Steady-State Kinetic Analysis:
Reactions were performed as described above. Quantification was done by scanning densitometry with a PhosphorImager (Typhoon Trio; GE Healthcare). The initial velocities of the reaction were calculated (Image Quant and GraphPad Prism 3.0) from the values of integrated gel band intensities: $I^*_T / I_{(T-1)}$.

where T is the target site, the template position of interest; I^*_T is the sum of the integrated intensities at positions T, T +1, . . . ,T + n.

All of the intensity values were normalized to the total intensity of the corresponding lane to correct for differences in gel loading. The apparent Km and kcat values were calculated by plotting the initial velocities in dependence of the nucleotide (dNTP) or primer (3'-OH) substrate concentrations and fitting the data according to Michaelis- Menten equation:
Kcat $(E)_0/(1 + Km/(S))$.

where $(E)_0$, was the input enzyme concentration and (S) was the variable substrate. Substrate incorporation efficiencies were defined as the k_{cat}/K_m ratio. Under single nucleotide incorporation conditions $k_{cat} = k_{pol}k_{off}/(k_{pol} + k_{off})$ and $K_m = K_d k_{off}/(k_{pol} + k_{off}$, where k_{pol} is the true polymerization rate, k_{off} is the dissociation rate of the enzyme–primer complex and K_d is the true Michaelis constant for nucleotide binding. Thus, kcat/Km values are equal to k_{pol}/K_d

Substrate concentrations used were 0.1–100 mM for dCTP or dATP and 5–300 nM for 3-OH primer ends.

Arabidopsis cell suspension culture and preparation of cell extracts:
Arabidopsis thaliana (ecotype Landsberg erecta) MM1

suspension cells were obtained from JAH Murray and grown as described *(Menges and Murray, 2002)*. Cell extracts were preparated as follows: MM1 cells pellet was lysed using a Hypotonic Buffer (10 mM TrisHCl pH 8, 1 mM EDTA pH 8, 5 mM DTT) added with a proteases inhibitor for 30 minutes in ice and homogenized by Dounce. Later, 0,5 volumes of a new buffer with high salt concentration were added to the extracts (50 mM TrisHCl pH 7.5, 1M KCl, 2 mM EDTA ph7.5, 2 mM DTT). The products were centrifuged for 3h at 13000 rpm (20000 g) in a TST60.4 rotor Sorvall Centrifuge, in order to recover the supernatant after eliminating the cellular waste. Then, the suspension was dialyzed over-night (O/N) in an hypertonic buffer (20 mM TrisHCl pH 8, 20% glycerol, 1 mM DTT, 100 mM NaCl), in order to obtain a more clear total crude extract. By mechanical and osmotic lysing processes, two total crude extracts were obtained: Crude extract A (CE A), from a $6x10^6$ cells/ml suspension collected in the stationary phase cells and the Crude extract C (CE C), was obtained from actively proliferating suspension cells ($1.8 X 10^6$ cells/ml).

Antibodies and proteins:
The antibodies against DNA pol λ (rabbit) were a kind gift of Prof. Ulrich Hübscher, University of Zürich-Irchel, Switzerland. Recombinant human PCNA was expressed and purified as described *(Maga et al., 2007)*.

Western Blotting assay for the DNA pol λ identification in MM1 cells:
CE protein content from MM1cells - previously separated by SDS-PAGE in 7.2% polyacrylamide gel electrophoresis- was transferred to a nitrocellulose membrane (Hybond, Amersham), by applying an electric field. Proteins were subsequently identified by a specific anti-human DNA pol λ antibody.

The membrane images showing bands corresponding to the different proteins were then detected by "Quanty One" software.

Immunoprecipitation of DNA pol λ from total crude extracts of MM1 cells:
The Protein A Sepharose beads were hydrated with a specific buffer for 30 minutes on ice, then centrifuged at 0.8 rpm for 5 minutes, the supernatant was then removed and two subsequent washes were performed by buffer RSB (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 2.5 mM $MgCl_2$). Further, samples were centrifuged again to remove the supernatant and the pellets were diluted in buffer A (1 mg/ml BSA, protease inhibitors) and shaked O/N at 4°C. After incubation, the samples were complementated with specific antibodies against the protein of interest (anti-DNA pol λ) and against a control antibody (anti-phosphatase-conjugated goat alkaline). After an hour of incubation, they were centrifuged to remove the supernatant and two washes were performed in buffer RSB. Subsequently, a defined amount of crude extracts (CE A and C) was added to each sample and incubated for 3 hours at 4°C. Then, the samples were centrifuged at 2000 rpm and put on ice. The supernatants thus obtained were separated from their beads, which were washed in RSB buffer. The new supernatants and their corresponding beads were finally separated by 7.5% SDS-PAGE and analyzed by western-blotting assays.

RESULTS

Human PCNA increases DNA polymerase activities in total crude extract (CE) assays from MM1 cells of *Arabidopsis thaliana*:
The plant DNA polymerase (DNA pol) activity was first evaluated, by performing enzymatic activity tests on two different samples of crude extracts from MM1 cells, corresponding to cells in active proliferation (CE C) or in the stationary phase (CE A).

Samples were incubated in the presence of poly (dA) oligo (dT), as a template primer and a radioactive nucleotide substrate (^{3}HTTP), in order to quantify the efficiency of the polymerization reaction. Under these conditions, we observed that with both extracts DNA pol activity (evaluated as pmol of (^{3}HTTP) incorporated) was a function of protein concentration. In particular, CE C showed a polymerase activity higher than that of CE A (Figure 1A). Interestingly, the subsequent addition of the human PCNA to all reaction mixtures increased the polymerization rate in both crude extracts (Figure 1B).

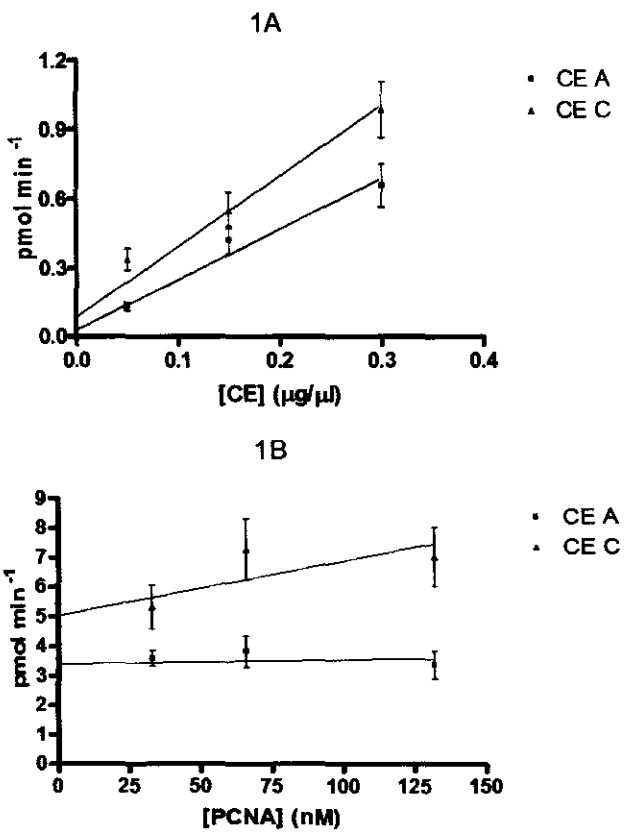

Figure 1: DNA polymerase activity in total crude extracts from *Arabidosis*. A) Variation of the polymerase activities in CE A and C from MM1 cells as a function of increasing amounts of crude extract (µg/µl of total soluble proteins). The polymerase activity is expressed in terms of pmol / min of product obtained. The squares represent the polymerase activity in the crude extract A (CE A), while the triangle indicates that of crude extract C (CE C). B) Effect of increasing concentrations of PCNA on the polymerase activities of CE A and C. The graph shows enzyme activity (pmol / min) as a function of increasing amounts of human PCNA (nM) added either to crude extract A (squares) or C (triangles) (µg/µl).

Crude extracts from MM1 suspension cells show preferential error-free dCTP incorporation opposite to 8-oxo-G, compared to dATP error-prone incorporation:

In vitro translesion assays were also conducted to measure the efficiency of synthesis of the *A. Thaliana* DNA pols in the presence of the 8-oxo-G oxidative lesion and, in particular, to estimate the dNTPs incorporation preference, while bypassing the oxidative lesion. In this regard, the process is referred to as error-free when the nucleotide substrate incorporated is dCTP and error-prone in the case of dATP insertion. An oligo-DNA substrate, lacking one base opposite to the 8-oxo-G site, was used in these assays (Figure 2). Gel sequencing analysis of the reaction products showed that both crude extracts had the ability to incorporate nucleotide opposite to the 8-oxo-G lesion. We observed a preferential utilization of dCTP (Lanes 4,5,10 and 11) with respect to the dATP substrate, that was incorporated in a smaller amount (Lanes 6,7 and 12). In addition, the increase of the signal seemed to be a function of the enzyme concentration in the crude extracts; this finding was significantly more marked for the crude extract C (Lane 5) compared to the crude extract A (Lane 12). To confirm the preferential correct incorporation of dCTP with respect to dATP, it was necessary to measure the catalytic rate for each substrate and to extrapolate the corresponding K_m and V_{max} values from the Michaelis-Menten equation. By comparing the K_m values obtained (Figure 3B), we observed a three-fold higher affinity for dCTP (K_m = 6.3 ± 0.5 mM) compared to dATP (K_m =13.5 ± 0.5 mM). The efficiency of incorporation, expressed by the V_{max} / K_m ratio, was also evaluated: dCTP incorporation appeared to be approximately three-fold higher than that of dATP. These data confirmed the hypothesis of a error-free translesion synthesis occurring opposite to the 8-oxo-G damage site.

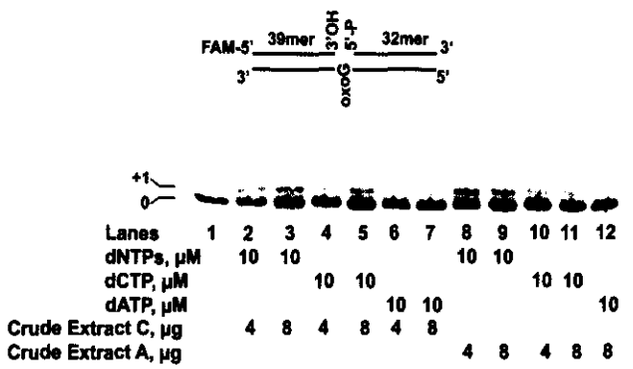

Figure 2: Nucleotide incorporation opposite to 8-oxo-G by crude extract A and C. The dsDNA substrate is shown at the top of the figure: It was incubated with dATP (Lanes 6,7 and 12), dCTP (Lanes 4,5,10 and 11) and dNTPs (Lanes 2,3,8 and 9) in the presence of CE C (Lanes 2,7) or CE A (Lanes 8,12). Lane 1 is the reaction control in the absence of both nucleotides and crude extract.

MM1 crude cell extracts incorporates dCTP opposite an 8-oxo-G with the same efficiency as opposite a normal (undamaged) G:

As expected on the basis of the DNA template sequences, we observed a dose-dependent dCTP incorporation opposite to the dGTP site (Figure 4A), while no incorporation was detected for dATP (not shown). The polymerase activities, evaluated for both control and damaged substrates, did not significantly differ: In fact, the K_m values calculated were, respectively 5.5 ± 0.5 mM (controls) and 6.5 ± 0.5 mM (damaged) (Figure 4B). Similarly, no significant differences emerged with regard to the V_{max} / K_m ratio between the control and the damaged templates.

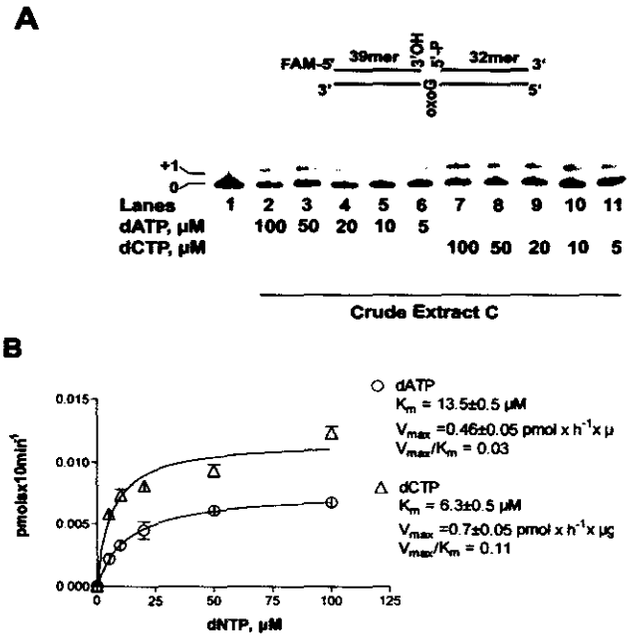

Figure 3: Kinetics of 8oxoG bypass in the CE C. A) The dsDNA substrate is shown at the top of the panel. CE C was incubated in the presence of 1nt gapped 8-oxo-G DNA substrates, with decreasing doses (100, 50, 20, 10 and 5µM) of dATP (lanes 2-6) or dCTP (lanes 7-11). In lane 1 is shown the control of the reaction, in the absence of both nucleotides and CE C. B) Polymerase activity (pmols/10min) in the CE C increases as a function of the concentration of dATP (circle) or dCTP (triangle). The legend indicates the K_m and V_{max} values for each nucleotide, as well as their corresponding V_{max}/K_m ratio.

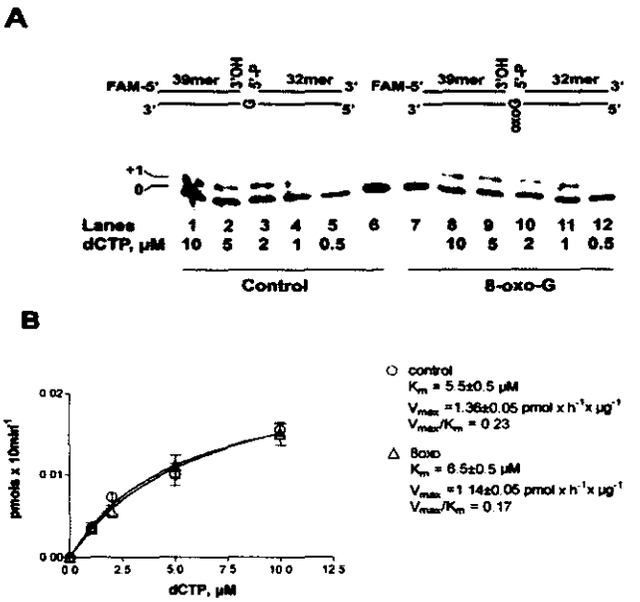

Figure 4: Incorporation of dCTP opposite to control and 8-oxo-G damaged substrate. A) The control and damaged dsDNA substrates shown in panel A were incubated with decreasing concentrations of dCTP: (lanes 1- 5) control substrate or (8-12) damaged substrate in the presence of CE C. Lanes 6 and 7 are the controls of the reactions that are FAM-labelled 39 mer primer. B) Polymerase activity (pmols/10min) increases as a function of dCTP in control (circle) or in a 8-oxo-G damaged (triangle) substrate. In the legend, the K_m and V_{max} values are indicated for each substrate as well as their corresponding V_{max}/K_m ratio.

Identification of DNA pol λ in MM1 cells:

Recent studies demonstrated that the mammalian DNA pol λ is a very efficient enzyme in the reparative mechanism, involved in the error-free translesion synthesis opposite to the 8-oxo-G damage site. In the light of these reports, it was interesting to verify whether this functional ability is also preserved in the plant cells.

In order to identify the presence of DNA pol λ in crude cell extracts of *Arabidopsis*, we used two different approaches: Western blotting and immunoprecipitation assays. As shown in Figure 5, the presence of a 60 kDa band with a good resolution was observed following immunodetection using a polyclonal antibody specific for the human DNA pol λ. This band size is consistent with that of a 529 aa polypeptide as deduced by cDNA sequence analysis. A 30 kDa band was also visible, likely corresponding to a degradation product of DNA pol λ (Figure 5).

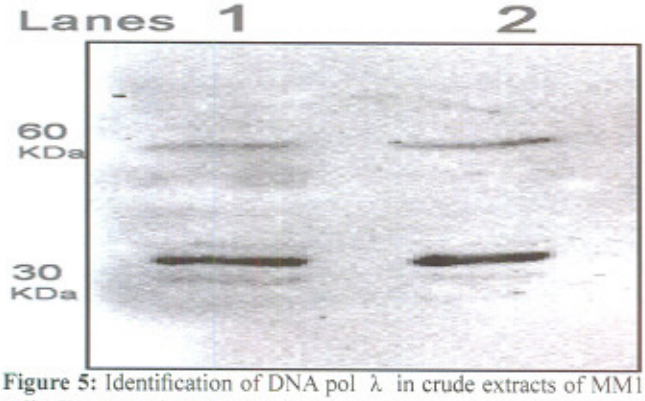

Figure 5: Identification of DNA pol λ in crude extracts of MM1 cells. Western Blotting assay shows the presence of the DNA pol λ in the CE A (lane 1) and CE C (lane 2) revealed by a specific anti-human DNA pol λ polyclonal antibody raised in rabbit.

immunoprecipitation of DNA pol λ:

For further confirmation of the results obtained, the crude extract was treated with antibodies against human DNA pol λ or, as a control, with unspecific IgGs. The immunoprecipitated complex was then rescued using Protein A Sepharose beads. Resulting pellet and supernatant fractions were analyzed using antibodies against human DNA pol λ. As shown in (Figure 6), this approach proved to be able to efficiently immunoprecipitate the enzyme from *Arabidopsis* crude extracts.

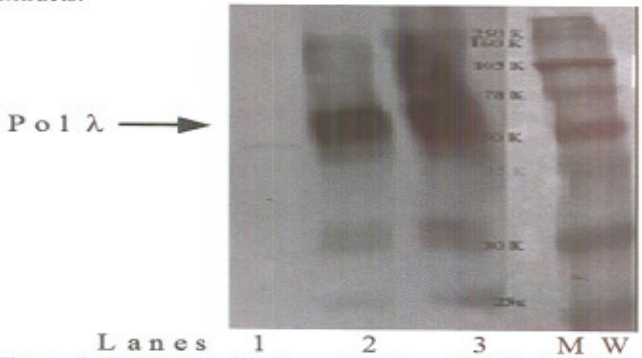

Figure 6: Immunoprecipitation of DNA pol λ. The Protein A Sepharose beads were incubated with CE treated with either control IgG (Lane1) or with anti- human DNA pol λ antibody (Lanes 2 and 3). Lane 1, precipitated proteins with control IgG. Lane 2, precipitated proteins with anti-human DNA pol λ antibody. Lane 3: input crude extract (1:10). Molecular weight markers were loaded on the right side of the gel.

DISCUSSION

The aim of this work was to understand, at the enzymatic and molecular levels, how *Arabidopsis* cells overcome 8-oxo-G damage using translesion DNA synthesis. This was obtained assaying total DNA polymerase activity in crude extracts (CE) of MM1 cells. DNA polymerase activity was higher in actively proliferating cells as compared to stationary phase ones. Such phenomenon increases further when human PCNA was added, suggesting that functional interaction between this protein and DNA pols occurs similarly in both animal and plant cells (Figure 1A).

Using translesion synthesis assays conducted by testing CE of *Arabidopsis* using an artificial substrate bearing 1 nt gap containing the 8-oxo-G damage, we have proved that both dATP and dCTP are incorporated. This result is a direct consequence of the intrinsic chemical and physical structure of the lesion 8-oxo-G. As reported in the literature (*Garcia-Ortiz et al., 2004*), if the structure of the sugar-phosphate backbone of the template DNA is kept rigidly into the active site of DNA polymerases, it favors the syn-conformation of the 8-oxo-G and the insertion of dATP on the complementary strand. On the contrary, if the template strand has the ability to position himself within the active site in order to accommodate the electronegative oxygen at C8, the 8-oxo-G anti-conformation may be permitted, favoring the correct insertion of dCTP opposite the lesion *(Kamiya, 2004)*. Therefore, the nucleotide incorporation opposite the 8-oxo-G damage mainly depends on the structural characteristics of the specific DNA polymerase involved.

The most significant results that have emerged by our study is the preferential incorporation of dCTP compared to dATP (Figure 4B). This is even more evident considering the V_{max} / K_m values calculated per individual nucleotides, from which it emerges a higher polymerase affinity (of CE) for the dCTP incorporation (approximately three times higher than of dATP). Moreover, dCTP incorporation was found similar opposite the normal base and 8-oxo-G damage, as shown by the polymerase activity values calculated for both substrates (8-oxo-G lesion and substrate control) which do not significantly differ between them (Figure 4B). Therefore, the preferential incorporation of the correct base reflects the presence of an highly efficient error-free translesion synthesis against the 8-oxo-G damage in extracts of plant cells. This situation is different from that found in protein extracts of animal cells, where the synthesis in the presence of 8-oxo-G is usually error-prone, with a dCTP:dATP incorporation ratio of 1:1-1:3. Other recent experiments have outlined that DNA pol λ is an efficient enzyme in the repair mechanisms of oxidative damage *(Maga et al., 2007)*. In light of this, it was interesting to consider whether DNA pol λ of *Arabidopsis* could play a significant role in bypassing 8-oxo-G and whether its function is similar to that of the corresponding animal enzymes.

With a preliminary analysis of CE of *Arabidopsis* it has been possible to identify DNA pol λ, by Western blotting

and immuneprecipitation analysis, as a polypeptide of molecular weight around 60 kDa (Figure 5A) similar to that of DNA pol λ of *Oryza sativa* (60.9 kDa) and of mammals (66 kDa).

In conclusion, with this study it was possible to show for the first time the ability of the plant cell to carry out the error-freebypassing of 8-oxo-G DNA lesion, through the intervention of translesion DNA pols that preferentially incorporate the correct nucleotide dCTP.

In particular, it has been possible to identify the presence of DNA pol λ in cell extracts of *Arabidopsis thaliana*, which has been shown to be the enzyme involved in error-free bypass of 8-oxo-G in animal cells. The ability to bypass oxidative lesion by the incorporation of a correct nucleotide will avoid the establishment of a DNA mutation, thus preserving the original genetic information of the plant cell. Preliminary experiments using a recombinant At DNA pol λ tend to confirm the biochemical data obtained in the course of this study. The evaluation of the over-expression of At DNA pol λ coding sequence is also under evaluation.

REFERENCES

Amoroso, A., Crespan, E., Wimmer, U., et al. 2008. DNA polymerases and oxidative damage: Friends or foes? Current Molecular Pharmacology **1**(2):162-170.

Babiychuk, E., Cottrill, P. B., Storozhenko, S., et al. 1998. Higher plants possess two structurally different poly(ADP-ribose) polymerases. The Plant Journal: For Cell and Molecular Biology **15**(5):635-645.

Bray, C. M. and West, C. E. 2005. DNA repair mechanisms in plants: Crucial sensors and effectors for the maintenance of genome integrity. New Phytologist **168**(3):511-528.

Britt, A. B. 1999. Molecular genetics of DNA repair in higher plants. Trends in Plant Science **4**(1):20-25.

Collins, A. R. 1999. Oxidative DNA damage, antioxidants and cancer. BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology **21**(3):238-246.

Crespan, E., Hübscher, U. and Maga, G. 2007. Error-free bypass of 2-hydroxyadenine by human DNA polymerase ë with proliferating cell nuclear antigen and replication protein A in different sequence contexts. Nucleic Acids Research **35**(15):5173-5181.

Garcia-Ortiz, M. V., Ariza, R. R., Hoffman, P. D., et al. 2004. *Arabidopsis* thaliana AtPOLK encodes a DinB-like DNA polymerase that extends mispaired primer termini and is highly expressed in a variety of tissues. The Plant Journal: For Cell and Molecular Biology **39**(1):84-97.

Kamiya, H. 2003. Mutagenic potentials of damaged nucleic acids produced by reactive oxygen/nitrogen species: Approaches using synthetic oligonucleotides and nucleotides: Survey and summary. Nucleic Acids Research **31**(2):517-531.

Kamiya, H. 2004. Mutagenicities of 8-hydroxyguanine and 2-hydroxyadenine produced by reactive oxygen species. Biological and Pharmaceutical Bulletin **27**(4):475-479.

Krahn, J. M., Beard, W. A., Miller, H., et al. 2003. Structure of DNA polymerase beta with the mutagenic DNA lesion 8-oxodeoxyguanine reveals structural insights into its coding potential. Structure (London, England) **11**(1):121-127.

Macpherson, P., Barone, F., Maga, G., et al. 2005. 8-oxoguanine incorporation into DNA repeats *in vitro* and mismatch recognition by MutSalpha. Nucleic Acids Research **33**(16):5094-5105.

Maga, G., Villani, G., Crespan, E., et al. 2007. 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Nature **447**(7144):606-608.

Menges M., Murray J.A. 2002. Synchronous Arabidopsis suspension cultures for analysis of cell-cycle gene activity. Plant J. **30**: 203-12.