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Repair of 8-oxoG is slower in endogenous nuclear genes than in mitochondrial DNA and is without strand bias

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Abstract

DNA is vulnerable to the attack of certain oxygen radicals and one of the major DNA lesions formed is 7,8-dihydro-8-oxoguanine (8-oxoG), a highly mutagenic lesion that can mispair with adenine. The repair of 8-oxoG was studied by measuring the gene specific removal of 8-oxoG after treatment of Chinese hamster ovary (CHO) fibroblasts with the photosensitizer Ro19-8022. This compound introduces 8-oxoG lesions, which can then be detected with the *Escherichia coli* formamidopyrimidine DNA glycosylase (FPG). In this report we present gene specific repair analysis of endogenous genes situated in different important cellular regions and also the first analysis of strand specific DNA repair of 8-oxoG in an endogenous gene. We were not able to detect any preferential repair of transcribed genes compared to non-transcribed regions and we did not detect any strand-bias in the repair of the housekeeping gene, dihydrofolate reductase (*DHFR*). In vivo, mitochondrial DNA is highly exposed to reactive oxygen species (ROS), and we find that the repair of 8-oxoG is more efficient in the mitochondrial DNA than in the nuclear DNA.

Keywords: DNA repair; Base excision repair (BER); 8-oxoG; Mitochondria; Transcription coupled repair (TCR)

1. Introduction

An inevitable form of cellular stress is the constant exposure to reactive oxygen species (ROS), which are formed as metabolic by-products and by exogenous sources such as γ-radiation. ROS are genotoxic and induce a variety of DNA lesions, including oxidized bases, abasic (AP) sites, and DNA strand breaks (reviewed in [1]). Unrepaired, oxidative lesions in DNA are likely to play an important role in carcinogenesis, aging, heart disease, cataracts, and brain dysfunction (reviewed in [2]). One of the most abundant oxidized base lesions is the mutagenic 7,8-dihydro-8-oxoguanine (8-oxoG), which can mispair with adenine and thereby introduce GC → TA transversions during replication [3].

Mammalian cells have several mechanisms for repairing damaged DNA. Two major pathways are base excision repair (BER) and nucleotide excision repair (NER). BER eliminates single damaged base residues and is the primary defense against oxidative damage. The initial step of BER is the removal of the aberrant base by a DNA glycosylase. Most DNA glycosylases remove several structurally different damaged bases, and some of them have overlapping substrate specificities, which indicates that they may

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serve as backup systems for each other (reviewed in [4]). The major mammalian DNA glycosylase responsible for removing 8-oxoG base-paired to cytosine from DNA is the 8-oxoguanine glycosylase 1 (OGG1) [5–8]. Different isoforms of the OGG1 protein are produced by alternative splicing, and the splice-form determines whether the protein-product is transported to the nucleus or to the mitochondria [9]. NER is the principle pathway by which mammalian cells remove UV damage and other helix distorting lesions from the nuclear DNA. It is well established that many lesions that are recognized and repaired by the NER pathway are repaired in a transcription coupled and strand specific manner (reviewed in [10]). The coupling between transcription and repair appears to require a functional Cockayne syndrome group B (CSB) protein [11].

Mitochondria are essential cellular organelles that contain their own DNA (mtDNA). An early report demonstrated the absence of repair of the common UV-lesions, cyclobutane pyrimidine dimers (CPDs), from mtDNA [12]. This lead to the notion, that mitochondria had no DNA repair capacity. However, more recent reports have described the repair of other types of DNA damage in the mitochondria, including oxidative base damage [13,14]. In addition, several BER enzymes have been isolated from mitochondria [15–18]. These reports demonstrate the existence of a mitochondrial BER pathway, but knowledge regarding the mechanisms of the repair process is still very limited. The majority of cellular oxygen is consumed by mitochondria. Thus, mtDNA is highly exposed to ROS due to the proximity to the process of oxidative phosphorylation [19]. A better understanding of BER in the mitochondria is very important since accumulation of oxidative lesions in mtDNA has been implicated in the cause of aging and several human diseases (reviewed in [2,20,21]).

In the current study, we examine the heterogeneity of 8-oxoG repair in nuclear and mitochondrial DNA. We have investigated the repair of 8-oxoG in different regions of the nuclear genome: the housekeeping gene dihydrofolate reductase (*DHFR*), the ribosomal RNA gene (*rDNA*), and a non-transcribed nuclear region located downstream of the *DHFR* gene. We have also measured the repair in mitochondrial DNA, which unlike nuclear DNA, is not packed into a condensed chromatin structure. Furthermore, we investigated whether there was strand specificity in the repair of 8-oxoG lesions in the actively transcribed *DHFR* gene. For introduction of 8-oxoG in genomic DNA of CHO cells in culture we used the photosensitizer Ro19-8022 (RO), which has been shown to primarily introduce 8-oxoG and is regarded as a model compound superior to other photosensitizers for introduction of oxidative DNA damage in cells [22]. The *Escherichia coli* enzyme formamidopyrimidine DNA glycosylase (FPG) was employed to detect the induced lesions. It excises 8-oxoG and formamidopyrimidine (FaPy) lesions and cleaves the DNA backbone by β,δ-elimination [23,24]. We performed the experiments using the CHO B11 cell line, which can be considered as a model cell line for investigating transcription coupled repair (TCR). Thus, it has been used in studies analyzing gene specific repair of a broad range of different lesions such as UV induced CPDs, heterocyclic amines, methylated and alkylated DNA adducts and cisplatin and psoralen induced inter- and intra-strand crosslinks [25–30]. Numerous repair studies have focused on TCR and so far we have based our assessments on studies done in endogenous genes. In that regard 8-oxoG is an exception as for this lesion no such studies are available. The difficulty has been to get a sufficient frequency of lesions introduced into the endogenous genes and this is where RO is unique. A recent study using a plasmid shuffle assay [31] suggested that 8-oxoG is removed by a CSB dependent TCR pathway. There are, however, significant differences between the repair mechanisms of lesions in endogenous genes and on plasmids introduced into cells [32], and we wanted to explore the former, since we believe it provides a better model of the in vivo situation.

2. Materials and methods

2.1. Cell culture

The Chinese hamster ovary (CHO) fibroblast cell line B11, which has the housekeeping dihydrofolate reductase (*DHFR*) gene and a downstream nontranscribed region amplified [33], was grown in Ham's F-12 medium without glycine, hypoxanthine and thymidine (Gibco), supplemented with 500 nM methotrexate (MTX) (Sigma), 10% dialyzed fetal bovine serum (Gibco) and penicillin/streptomycin.

Prior to treatment with the DNA-damaging agent, cells were replated in 150 mm dishes (for gene specific repair assays) or 100 mm dishes (for RNA synthesis recovery assays) and grown to 80% confluence.

2.2. Introduction of oxidative DNA base damage in cells in culture

Exponentially growing B11 cells were incubated with RO (250 μM, OD_{425} = 0.4) in phosphate buffered saline (PBS) with glucose (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 1% glucose, pH = 7.4) at 37 °C for 1 h. The RO treated cells were placed on ice and exposed to visible light from a 1000 W halogen lamp at a distance of 15 cm for 3–7 min. Under dim yellow light the cells were then washed twice with cold PBS and lysed (in 0.5 M Tris, pH = 8, 20 mM EDTA, 10 mM NaCl, 1% SDS and 0.5 mg/ml proteinase K) or allowed to repair-incubate in the media described above for various periods of time before lysis and determination of DNA damage.

2.3. Gene specific repair

This assay was adapted from that described in detail elsewhere [34]. Briefly, after DNA damage induction and repair incubation, cells were lysed and treated with proteinase K. Genomic DNA was isolated by salt extraction [35], followed by RNase treatment and incubation with restriction endonuclease *Kpn*I. DNA was ethanol precipitated, resuspended in TE buffer, and the concentration was measured spectrophotometrically at 260 nm.

In the first biological experiments the repair incubation was done in the presence of 10^{-5} M bromodeoxyuridine and 10^{-6} M fluorodeoxyuridine and subsequently the replicated DNA was separated from non-replicated DNA on CsCl gradients. However, no significant replication was measurable within the 8 h of repair-incubation and this step was therefore omitted in subsequent experiments.

From each timepoint 1 μg of DNA was either mock-treated or treated with 3 ng of FPG for 1 h at 37 °C in FPG reaction buffer (70 mM Hepes-KOH, pH = 7.6, 100 mM KCl, 0.5 mM EDTA, 5 mM β-mercaptoethanol and 5% glycerol). Subsequently, all samples were treated with 0.1 M NaOH for 30 min at 37 °C to cleave the DNA strands at AP sites. Finally, alkaline loading dye was added to the samples before electrophoresis. The DNA samples were then electrophoresed overnight at 25 V in a 0.6% agarose gel under alkaline conditions. DNA was transferred to a Hybond N+ membrane (Amersham Pharmacia Biotech) using standard protocols.

Before hybridization with DNA probes the membranes were incubated in prehybridization buffer (0.342 M Na_2HPO_4, 0.088 M NaH_2PO_4, 7% SDS, 2 mM EDTA, pH = 7.2) for at least 30 min. DNA probes, prepared as described below, were added and the membranes were hybridized with the probe over night at 68 °C. For RNA-probes the membranes were prehybridized for 24 h in Hybrisol I® (Intergen). The RNA probes, prepared as described below, were added to the membrane in fresh hybridization solution and the membranes were hybridized at 45 °C for 24 h.

Non-hybridizing probe was removed by stringent washes and the blots were visualized by autoradiography using a Personal Molecular Imager® (BioRad). Quantification of the bands was done, by applying Quantity One® (BioRad) software. The number of lesions per restriction fragment were determined by calculating the ratio of full-sized restriction fragments in the lane with FPG-treated DNA to the density of the corresponding untreated sample and then applying the Poisson distribution. The gene specific repair was expressed as percent repair. Deprobing of the membranes were done by treatment with 0.4 M NaOH for 30 min and subsequent incubation in 0.1X SSC, 0.1% SDS and 0.2 M Tris–HCl, pH = 7.5 for 15 min at 45 °C.

2.4. Probes

Double-stranded DNA probes were prepared with a random-primed labeling kit (Amersham Pharmacia Biotech). The pMB5 probe was used for the coding region of the *DHFR* gene as previously described [36]. This probe is a 3.4 kb fragment of the 5′-transcribed end of the *DHFR* gene and it recognizes a 14 kb fragment of *Kpn*I digested CHO DNA. The probe for the non-transcribed nuclear region was CS14 [36], which recognizes a 14 kb non-transcribed region localized just downstream of the *DHFR* gene. The probe used for the *rDNA* was the pA_{BB} probe [37,38], which has a 1.4 kb insert of human 28S *rDNA*. The probe is homologous to the CHO *rDNA*, and it detects a

9 kb fragment of *Kpn*I digested CHO DNA. The *Kpn*I fragment of mitochondrial DNA is recognized by the pCRII probe [13] and spans approximately 16 kb. The insert in the pCRII probe is 2.574 kb and covers the ribosomal region of the mitochondrial DNA.

The strand specific RNA probes were synthesized using the pZ3d8 plasmid described in detail elsewhere [26]. The plasmid was digested with *Bam*HI when using T7-RNA polymerase and with *Kpn*I when using SP6 RNA polymerase to generate templates for strand specific riboprobes. Reactions with T7 and SP6 RNA polymerases to produce transcribed and non-transcribed strand probes, respectively, were conducted using 2 μg of digested plasmid DNA template per reaction and were carried out with [^{32}P] CTP using the Boehringer Mannheim SP6/T7 transcription kit and protocol. After transcription reactions, radiolabeled riboprobes were purified using the RNeasy Mini Kit® (Qiagen) and subsequently added to the membrane.

2.5. Cell survival

Survival of cells after exposure to photoactivated RO was determined by trypan blue (Gibco) exclusion. Cells were grown to 80% confluence. Cells exposed to RO and light were incubated with RO for 1 h as described above, exposed to 6 min of light, and then allowed to recover for 8 h in the medium described above. As control experiments cells were treated with RO and no light or without treatment at all. Cells were then trypsinized and mixed with 0.2% trypan blue. The number of total cells and the number of blue cells were counted.

2.6. RNA synthesis recovery

Exponentially growing cells were incubated with RO (as described above) for 1 h and then exposed to 6 min of light. Cells were then allowed to repair in normal growth medium for 4 or 8 h. As control experiments cells were either mock treated or incubated with 5 μg/ml Actinomycin D for 4 or 8 h. One hour prior to each timepoint the medium was replaced by fresh medium containing 5 μCi/ml [^{3}H] uridine (Amersham Pharmacia Biotech) to pulse-label total RNA. After 1 h, cells were washed twice with PBS and lysed in 150 mM NaCl, 0.5% SDS, 5 mM Tris, pH = 8.0, 1 mM EDTA and 0.1 mg/ml proteinase K. Ten percent ice-cold trichloroacetic acid (TCA) was added to the labeled samples and the samples were subsequently incubated over night at 4 °C. The lysates were then spotted onto GF/C Whatman glass filters, washed twice with 5% TCA, once with 70% ethanol and finally with acetone, dried and counted in a liquid scintillation counter. In order to adjust for possible differences in number of cells after different treatments, the number of cells from experiments run in parallel to the experiments for the RNA synthesis measurement was counted after 4 and 8 h, respectively.

3. Results

3.1. Introduction of 8-oxoG in DNA in vivo

For detection of 8-oxoG lesions in DNA isolated from RO-damaged CHO cells we used the *E. coli* FPG enzyme [13]. To optimize the FPG protein detection of 8-oxoG lesions, DNA from RO treated cells and mock treated cells was incubated with various amounts of FPG, electrophoresed, blotted and probed for *DHFR*. The specific cleavage by FPG at RO induced lesions was calculated by subtracting the number of FPG sensitive sites (FSS) in DNA from mock treated cells, which equals unspecific cleavage, from the number of FSS in DNA from RO treated cells. It is noted that specific cleavage by FPG at RO induced lesions reaches a plateau, when incubating with 3 ng FPG or more (Fig. 1A). The optimal FPG concentration was, therefore, found to be 3 ng FPG per microgram of DNA, which resulted in highly specific cleavage of DNA from RO treated cells, but no cleavage of DNA from mock treated cells. In order to identify conditions where RO would induce a sufficient level of 8-oxoG lesions, CHO B11 cells were treated with different concentrations of RO (Fig. 1B) and exposed to light for various periods of time (Fig. 1C), respectively. Together, these dose responses identify conditions where a high level of FSS is induced by RO plus light and where all the induced FSS are specifically recognized by FPG. Treating the cells with 250 μM RO followed by 6 min of light exposure induces ≈0.7 8-oxoG lesions per 14 kb fragment in the actively transcribed *DHFR* gene, which subsequently are detected by incubation with 3 ng FPG (Fig. 1).

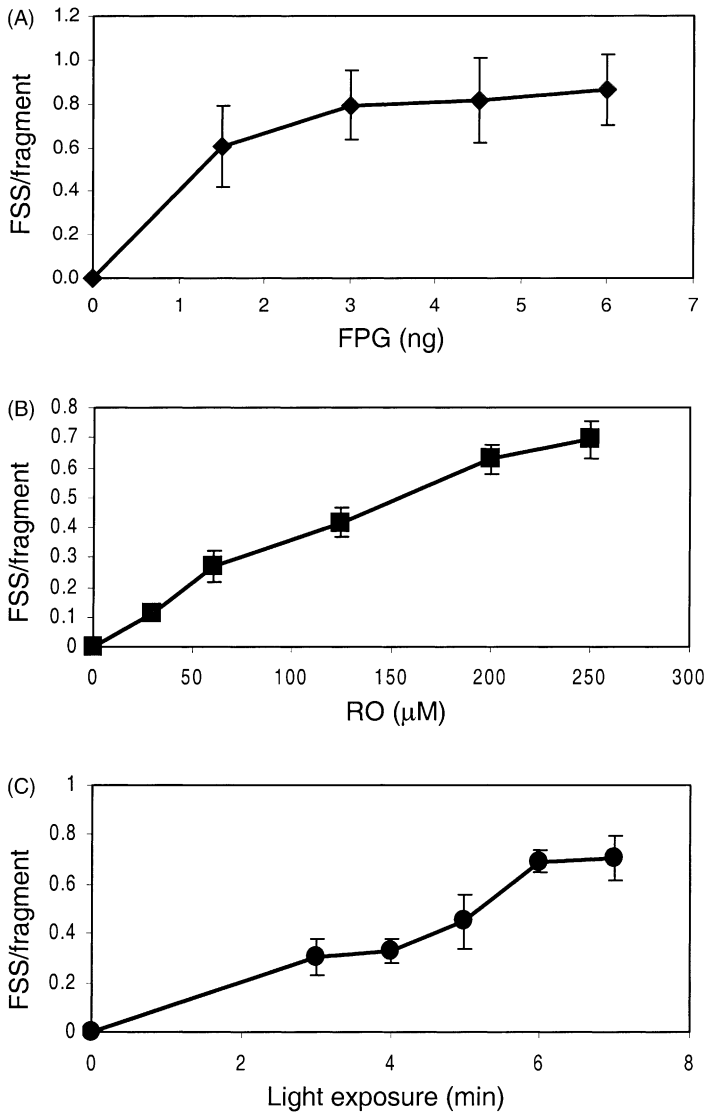

Fig. 1. Introduction of FPG sensitive sites (FSS) in the *DHFR* gene of cells in culture. (A) Specific FPG cutting of RO induced DNA damage. Diagram shows the number of FSS in DNA from cells treated with 250 μM RO and exposed to 6 min of light after unspecific FPG cutting of DNA from mock treated cells has been subtracted; (B) the number of initial FSS in the DNA as function of concentration of RO. The cells were incubated with various concentration of RO for 1 h and exposed to light for 6 min; (C) initial lesion frequency as function of light exposure. The cells were incubated with 250 μM RO for 1 h and then exposed to light for various periods of time. The values in A, B and C, respectively, are the average of four to five experiments and error-bars represent standard deviation.

We next investigated the formation and repair of RO induced lesions in four different regions of the genome: the *DHFR* gene, the *rDNA*, a transcriptionally silent region and the mitochondrial genome, which, unlike the nuclear DNA, has no chromatin structure. The genomic regions examined and the probes used are illustrated in Fig. 2. Different RNA polymerases are responsible for the transcription of

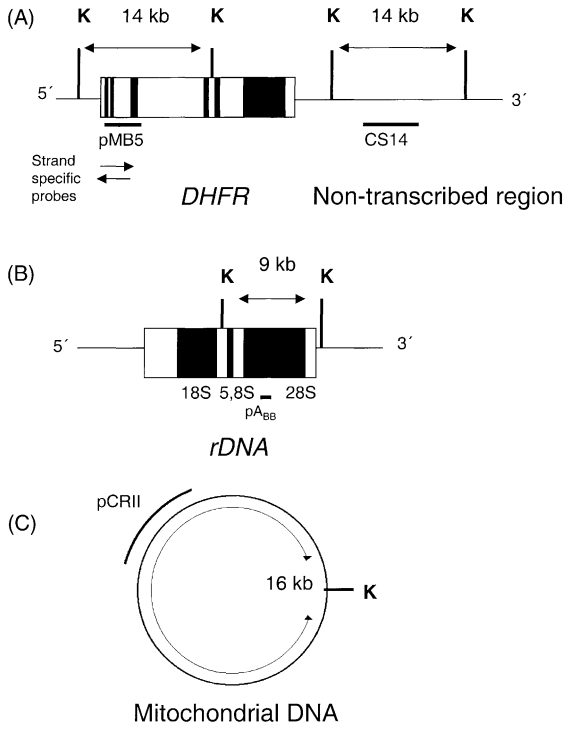

Fig. 2. Regions of the CHO genome analyzed in this study. K: *Kpn*I restriction sites. Horizontal bars below the gene-maps indicate the probes used. (A) The *DHFR* gene, probed for by pMB5 and the downstream non-coding region, probed for by the CS14 probe; (B) the *rDNA*, where the pA_{BB} probe recognizes a 9 kb fragment containing the 5.8S and 28S region of *rDNA*; (C) the probing of the mitochondrial DNA by pCRII visualizes a 16 kb fragment. The map is based on the sequence known for guinea pig mitochondrial genome.

the genes. RNA polymerase I is responsible for the transcription of *rDNA*, RNA polymerase II is responsible for transcription of the *DHFR* gene, while mitochondrial transcription is via a proprietary polymerase. Additionally, the *rDNA* is rich in guanine and has a different nuclear localization than the *DHFR* gene, which may influence the removal of damage from that particular nuclear region. In the four regions of the CHO genome investigated here, the lesion frequency after RO plus light treatment was on average 0.5 ± 0.07 8-oxoG/10 kb (Table 1). Thus, the base lesions induced by RO appear to be evenly distributed in the different regions of the genome. This allows us to compare the repair of 8-oxoG in these four regions of intracellular genomic DNA.

To ensure that treatment of the cells with RO did not cause extensive cellular death, cell viability was determined by trypan blue exclusion (Table 2). Mock treated cells were 99% viable while treatment with RO without light exposure resulted in 98% viability. After treatment with 250 μM RO and exposure to 6 min of light, approximately 73% of the attached cells were able to exclude trypan blue after 8 h of repair incubation. This suggests that the conditions used for introduction of 8-oxoG into the genomic DNA were not overtly toxic to the cells and, thus, in a physiological range.

3.2. Repair of 8-oxoG in different regions of the genome

We next compared the repair kinetics in the various regions of the genome, using different probes covering the transcribed genes, *DHFR* and *rDNA*, a non-transcribed region (CS14) and the mtDNA, respectively (Fig. 3A). In all genomic regions examined, a significant level of repair of the RO induced base lesions was observed in the 8 h repair period investigated. After 8 h, 32% of the 8-oxoG lesions were repaired in the *DHFR* gene, 34% of the 8-oxoG lesions were repaired in the *rDNA*, and 28% of the 8-oxoG lesions were repaired in the non-transcribed DNA region (probed for by CS14) (Fig. 3B). As depicted in

Table 1
Initial lesion frequency in different regions of the genome

Genomic region	8-oxoG per fragment[a]	Size of fragment (kb)	8-oxoG per 10 kb
DHFR	0.67 ± 0.10	14	0.48
CS14	0.65 ± 0.05	14	0.46
rDNA	0.43 ± 0.10	9	0.48
Mitochondria	0.84 ± 0.13	16	0.53

[a] The data represents the average ± standard deviation of five independent biological experiments with data from two to four gels per experiment.

Table 2
Viability of CHO cells determined by trypan blue exclusion

Treatment[a]		Total number of cells after treatment	Cells not stained after treatment (%)[b]
RO	Light		
−	−	1.4×10^7	99.4
+	−	1.4×10^7	98.4
+	+	1.1×10^7	72.6

[a] Cells treated with RO plus light were allowed to repair incubate for 8 h.

[b] Experiments were performed in duplicate.

Fig. 3B, the repair of 8-oxoG in the three different nuclear regions had nearly identical kinetics, whether the damage was localized in non-transcribed DNA or DNA transcribed by RNA polymerase I or RNA polymerase II.

We find that the repair rate for mtDNA was higher than that observed for nuclear DNA. After 6 and 8 h of repair 40 and 45%, respectively, of the RO induced lesions were repaired, which is significantly more than in any of the three nuclear regions investigated (Fig. 3). Despite the fact, that overall replication was shown to be inhibited in the 8 h repair period investigated

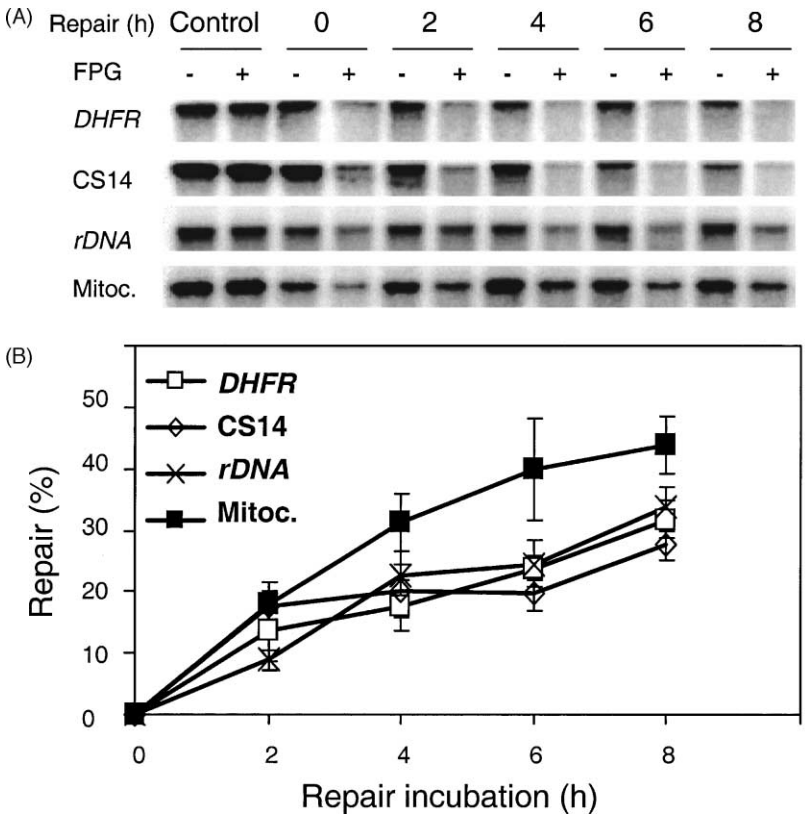

Fig. 3. Repair of 8-oxoG in the actively transcribed *DHFR* gene, a downstream noncoding region (probed for by CS14), the 28S part of the *rDNA*, and the mitochondrial genome was measured. *Kpn*I digested genomic DNA (1 μg) from RO treated cells, and mock treated cells (control), was isolated at various timepoints after damage induction as described in Section 2. Before Southern blot analysis DNA was either mock treated or treated with FPG (−/+). (A) Representative autoradiograms of Southern blots. *DHFR*: the actively transcribed *DHFR* gene probed for by the pMB5 probe. CS14: the non-transcribed region downstream of the *DHFR* gene probed for by the CS14 probe. *rDNA*: the 5.8S and 28S region of the *rDNA* probed for by pA_{BB}. Mitoc.: the mitochondrial genome probed for by pCRII; (B) quantification of the bands in the Southern blots presented as percent repair of photoactivated RO induced 8-oxoG in the four different regions of the genome investigated. The data presented are the average ± standard deviation from five different biological experiments with several gels from each experiment.

after RO treatment (Section 2), we wanted to exclude the possibility that the faster repair in the mitochondria could be due to a different rate of replication of mtDNA than of nuclear DNA. It has previously been demonstrated that mtDNA segregates similar to nuclear DNA in CsCl gradients [39] and we saw no difference in results obtained with or without such gradients (Table 3). Furthermore, when we analyzed the amount of mtDNA relative to the amount of nuclear DNA at all timepoints after damage induction, we found no increase, i.e. the level of mtDNA compared to nuclear DNA in repair experiments performed both with and without CsCl gradients remained constant (Table 3). This demonstrates that the observed faster repair of mtDNA is not a result of differential replication. Our results indicate that mitochondria are indeed very capable of

Table 3
Replication of mtDNA and possible effect on repair

Time after RO (h)	mtDNA relative to nuclear DNA[a]		Repair (%)[b]	
	+CsCl	−CsCl	+CsCl	−CsCl
0	1.00	1.00	0	0
2	0.91	0.87	16.5	21.8
4	1.00	0.90	36.6	28.8
6	0.94	0.96	39.3	40.7
8	0.98	0.95	45.6	43.8

[a] The amount of mtDNA relative to the amount of nuclear DNA measured at each timepoint after RO treatment, in experiments where supposable replicated DNA was separated (+CsCl) or not separated (−CsCl) from parental DNA using CsCl gradients. The ratio of mtDNA to nuclear DNA was set to 1 at timepoint 0 h.

[b] The repair of mitochondrial DNA at each timepoint after RO treatment in experiments where replicated DNA was either separated (+CsCl) or not (−CsCl) from parental DNA using CsCl gradients.

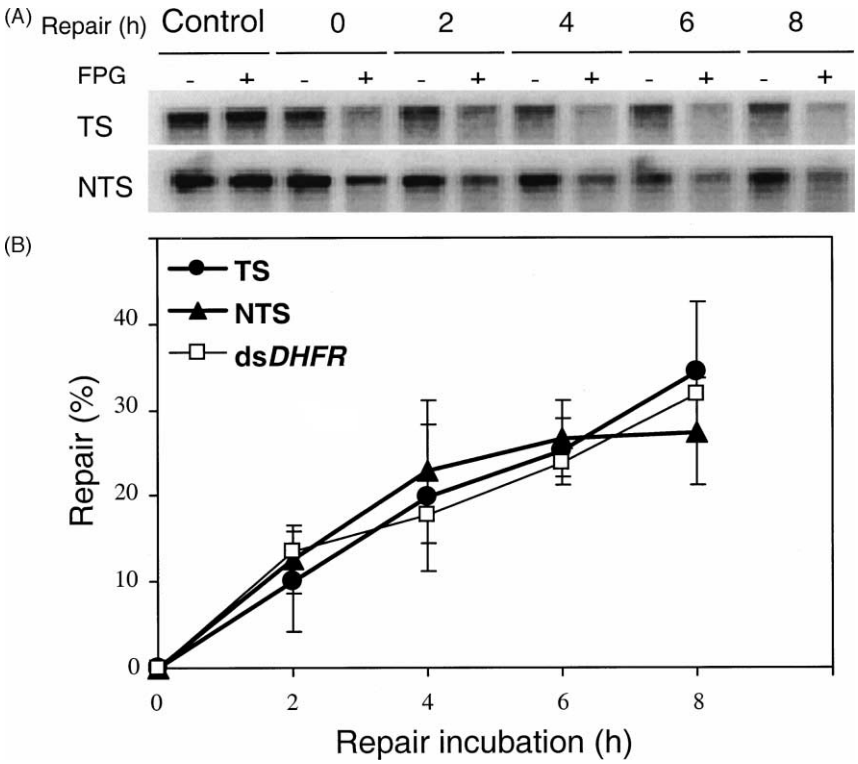

Fig. 4. Repair of 8-oxoG in the transcribed strand (TS) and the non-transcribed strand (NTS) of the actively transcribed *DHFR* gene. (A) Representative autoradiograms of Southern blots, prepared as described in Section 2 and in Fig. 3; (B) quantification of the bands in the Southern blots presented as percent repair of photoactivated RO induced 8-oxoG. The data presented are the average ± standard deviation from four different biological experiments with several gels from each experiment. The thin line indicates the ds*DHFR* repair also shown in Fig. 3.

Table 4
RNA synthesis recovery after treatment with RO + light

Treatment of cells	Time after RO exposure (h)	[^{3}H]uridine[a] (counts/cell $\times 10^{-2}$)	Relative RNA-synthesis (%)
Control	4	9.1 ± 1.3	100 ± 14.6
RO	4	5.7 ± 0.5	65.5 ± 5.8
Actinomycin D	4	0.8 ± 0.5	8.5 ± 5.9
Control	8	7.8 ± 1.3	100 ± 16.5
RO	8	6.1 ± 0.7	73.3 ± 8.8
Actinomycin D	8	0.5 ± 0.1	6.5 ± 1.8

[a] The data represents the average ± standard deviation of four experiments.

processing 8-oxoG lesions efficiently, which contributes to protection of the mitochondrial genome from the endogenous attack by ROS.

3.3. Repair of 8-oxoG in the individual strands of the actively transcribed DHFR gene

Our results so far, suggest that there is no preferential repair of the RO induced base lesions in the nuclear DNA, since active genes and bulk DNA were repaired with similar kinetics. Subsequently, we wanted to investigate whether there was strand specific repair of 8-oxoG in the actively transcribed *DHFR* gene. Strand specific repair is most reflective of TCR. Using strand specific RNA probes the repair kinetics of the two individual strands were analyzed. The initial lesion frequency in each strand of the *DHFR* gene was 0.70 ± 0.25 FSS per fragment for the transcribed strand and 0.71 ± 0.20 FSS per fragment in the non-transcribed strand, which corresponds to the initial lesion frequency measured by dsDNA probes in the *DHFR* gene. As shown in Fig. 4, there was no difference in the measured repair rates of the two strands of the *DHFR* gene. The repair rates of the transcribed strand and the non-transcribed strand were almost identical, which suggests that 8-oxoG lesions are not repaired in a strand specific manner, when localized in the endogenous *DHFR* gene of the CHO genome.

3.4. RNA synthesis recovery after RO treatment

One possible reason for the lack of TCR of the RO induced lesions could be if the RO treatment somehow prevented the cells from transcribing DNA. To test this possibility we pulse-labeled RNA with [^{3}H]uridine after treatment with RO and measured newly synthesized RNA by scintillation counting after repair incubation. To standardizethe [^{3}H]uridine quantitation, we counted the total number of cells from which RNA was isolated. Our results demonstrate that RO treated CHO cells are indeed capable of transcribing the DNA (Table 4). Thus, 4 h after RO plus light treatment the cells retained 65% transcription compared to mock treated cells, and 8 h after damage induction the transcription level was 73% of that in the mock treated cells. As a control experiment, we treated cells with Actinomycin D, which specifically inhibits transcription [40]. As expected we detected almost no labeled RNA from the actinomycin treated cells (Table 4).

4. Discussion

In this study, we were able to introduce 8-oxoG lesions into the genome of cultured CHO cells in vivo and follow the repair over time. Using photoactivated RO, approximately 1 FPG sensitive site per 20 kb was introduced and the lesions were distributed equally within the nuclear and mitochondrial genome. It has previously been shown that approximately 70% of the RO induced lesions are 8-oxoG [22], and therefore, this was by far the most abundant of the lesions formed in our experiments. After RO treatment the cells were viable and demonstrated efficient repair of the induced lesions. About 30% of the induced lesions in nuclear DNA and 45% of the induced lesions in mitochondrial DNA were repaired within 8 h. Furthermore, transcription in the RO treated cells was not inhibited profoundly after RO treatment and we, therefore, believe that we have a valid assay for investigating gene specific as well as TCR of 8-oxoG lesions positioned in endogenous genes.

When comparing three different regions of nuclear DNA, namely, the *DHFR* gene, the *rDNA* and a silent

region of bulk DNA (CS14), no difference in the repair rates of 8-oxoG was observed. Thus, no preferential repair of open transcribed genes over a condensed non-transcribed region was detected. These three regions have previously been shown to exhibit very different repair rates for the common UV-lesions, CPDs. Repair of CPDs in the *DHFR* gene was shown to be much faster than in *rDNA*, while hardly any repair of CPDs was detectable in the non-transcribed region [36,41]. The differences in repair rates for CPDs are primarily caused by differences in TCR, where the RNA polymerase II transcribed *DHFR* gene has very efficient TCR and the two other regions have no TCR of CPDs. When we investigated the repair of 8-oxoG in the individual strands of the actively transcribed *DHFR* gene, no preferential repair of the transcribed strand over the non-transcribed strand was observed and, therefore, we observe no indication of TCR of 8-oxoG. Hence, our results for repair of 8-oxoG localized in endogenous genes do not verify the recently published papers by Le Page and coworkers, which propose a TCR mechanism for the repair of 8-oxoG [31,42]. In order to introduce and observe the repair of 8-oxoG, these authors used single lesion plasmids carrying an 8-oxoG modification. Thus, they investigated repair of 8-oxoG in exogenous plasmids, whereas we have investigated repair of 8-oxoG in endogenous genes, where the context of chromatin structure is present. Previous work has shown that certain lesions are repaired very differently in episomal DNA compared to endogenous genes. For example Dean et al. [32] showed that 8-methoxypsoralen intra- and inter-strand adducts were removed much slower from a transcribed plasmid that from the endogenous *DHFR* gene [32]. In some cases the repair of damage in shuttle vectors may give a representative measurement of repair in endogenous genes, however, in others it may not. In the case of 8-oxoG, the data for plasmid repair and endogenous gene repair do not seem to correlate. However, it must be noted that we have performed our experiments using a CHO cell line whereas Le Page and coworkers used human cell lines and mouse embryonic fibroblasts [31,42].

Gene specific repair analysis of the oxidative lesion thymine glycol (Tg), which primarily is repaired by BER, have shown that Tg is repaired faster when located on the transcribed strand of the metallothionein IIA gene (*MTIIA*), than when located on the non-transcribed strand [42–44]. However, Tg induces significant distortion of the DNA double helix unlike most other oxidative base lesions or AP-sites [45]. Furthermore, it blocks the progression of both the DNA replication [46] and transcription by the T7 RNA polymerase [47]. However recently, it was demonstrated that Tg positioned in the transcribed strand in an in vitro system has no detectable effect upon transcription by mammalian RNA polymerase II [48]. It has been shown that the 8-oxoG lesion by itself does not alter the DNA helix structure significantly. Furthermore, 8-oxoG is highly mutagenic and, therefore, likely to be easily passed by DNA polymerases [3]. Whether 8-oxoG is capable of stalling the RNA polymerase, which is necessary for TCR to occur, has been a subject of debate. In vitro experiments have shown that 8-oxoG is easily passed by the *E. coli* RNA polymerase [49], but transfection of an 8-oxoG containing shuttle-vector into a repair deficient cell line showed that there was no transcription bypass of the 8-oxoG lesion [31]. It is possible that a factor other than OGG1 is capable of binding to the 8-oxoG lesion and thereby mediate transcription arrest. It seems obvious to speculate that such a factor could have much -nobreakgreater accessibility to an 8-oxoG lesion positioned in an exogenous plasmid, than to 8-oxoG in endogenous genes that are protected by histones.

From our present study in hamster cells, we can not exclude the possibility that there is a backup mechanism capable of repairing 8-oxoG in transcribed regions. The difference observed in repair rates of transcribed strands versus non-transcribed strands are very small when using the plasmid shuffle assay [31,42], and it could be that our assay is not sensitive enough to detect such a small difference. However, it seems clear that BER of 8-oxoG in WT cells is much more uniform in the repair of nuclear DNA, than NER of UV-lesions. Thus, NER is dependent on assembly of a large complex of many proteins and, therefore, dependent on a relatively open chromatin structure, while BER probably operates one protein at a time passing the damage on to the next enzyme in the pathway, like a baton [50].

The spatial proximity to the process of oxidative phosphorylation renders mitochondrial DNA highly exposed to ROS. DNA repair of oxidative DNA damage in the mitochondria is therefore very important for maintenance of mitochondrial homeostasis. If left

unrepaired, oxidative mtDNA lesions can cause mitochondrial dysfunction, a phenomenon observed in various diseases and aging (reviewed in [2,20,21]). It was earlier shown that 8-oxoG is repaired uniformly throughout the mitochondrial genome, with no preferential repair in heavily transcribed regions [13]. We find that the repair of the RO induced oxidative lesions is faster in the mitochondria than in the nuclear DNA. The faster repair of oxidative lesions in the mitochondria can be the result of a variety of differences between the nucleus and the mitochondria. First, the mtDNA is a closed circular molecule that unlike nuclear DNA is free of histones. One could presume that the repair enzymes would have greater accessibility to the lesions in this DNA than in highly condensed nuclear DNA. Secondly, the BER proteins in the mitochondria and the nucleus are not identical. Alternative splicing of pre-mRNA gives rise to at least two isoforms of OGG1, which are transported to the two different cellular compartments, respectively. Both isoforms of OGG1 have glycosylase and AP-lyase activity, but they differ in their C-terminus [9] and it is, therefore, possible that the activity varies between them. Thirdly, the concentration of active enzyme in the different compartments of the cell may differ. Future studies of the BER process in the mitochondria are likely to enlighten some of the similarities and differences in the BER process between the two cellular compartments.

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