UVA irradiation induces relocalisation of the DNA repair protein hOGG1 to nuclear speckles

Anna Campalans1,*, Rachel Amouroux1,*, Anne Bravard1, Bernd Epe2 and J. Pablo Radicella1,‡
1Département de Radiobiologie et Radiopathologie, Commissariat à l’Energie Atomique, UMR 217 CNRS/CEA, 18 route du Panorama, 92265 Fontenay aux Roses, France
2Institute of Pharmacy, University of Mainz, 55099 Mainz, Germany
*These authors contributed equally to this work
‡Author for correspondence (e-mail: jpradicella@cea.fr)

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Summary

The DNA glycosylase hOGG1 initiates base excision repair (BER) of oxidised purines in cellular DNA. Using confocal microscopy and biochemical cell fractionation experiments we show that, upon UVA irradiation of human cells, hOGG1 is recruited from a soluble nucleoplasmic localisation to the nuclear matrix. More specifically, after irradiation, hOGG1 forms foci colocalising with the nuclear speckles, organelles that are interspersed between chromatin domains and that have been associated with transcription and RNA-splicing processes. The use of mutant forms of hOGG1 unable to bind the substrate showed that relocalisation of hOGG1 does not depend on the recognition of the DNA lesion by the enzyme. The recruitment of hOGG1 to the nuclear speckles is prevented by the presence of antioxidant compounds during UVA irradiation, implicating reactive oxygen species as signals for the relocalisation of hOGG1. Furthermore, APE1, the second enzyme in the BER pathway, is also present in nuclear speckles in UVA-irradiated cells. The recruitment of DNA repair proteins to nuclear speckles after oxidative stress implicates these organelles in the cellular stress response.

Key words: Base excision repair, hOGG1, Nuclear speckles, Reactive oxygen species, UVA

Introduction

Cellular DNA is continuously exposed to metabolic and environmental agents that can damage it and thus threaten genome stability. Different DNA repair pathways have evolved and become specialised for particular DNA lesions. The main pathway responsible for the elimination of modified bases is base excision repair (BER), which is initiated through recognition and excision of the altered base by a specific DNA glycosylase. After oxidative stress, one of the main base lesions formed in the DNA is 8-oxoguanine (8-oxoG), which if left unrepaired leads to the accumulation of mutations. The major DNA glycosylase responsible for the removal of 8-oxoG in eukaryotic cells is hOGG1. One open question is how hOGG1 and other proteins initiating DNA repair manage to find and repair the damaged DNA, especially considering the high degree of DNA condensation in the cell nucleus (Halford and Marko, 2004; Banerjee et al., 2005).

The nucleus is far from being a homogeneous compartment. Instead, it is organised into domains. Proteinaceous nuclear bodies such as nucleoli, Cajal bodies and nuclear speckles are interspersed between both euchromatin and heterochromatin domains. Although the functions of most nuclear structures remain unknown, proteins implicated in different pathways are distributed in these specific compartments (Cremer and Cremer, 2001; Misteli, 2005; Handwerger and Gall, 2006). Different DNA processes such as replication, transcription and repair often take place in defined nuclear regions. There is now a consensus on the fact that coordination of these processes is highly dependent on components of nuclear architecture (Stein et al., 2003). It is generally accepted that polymerases involved in replication and transcription are organised into ‘factories’ and immobilised on the nuclear matrix, which provides architecturally organised nuclear microenvironments (Cook, 1999; Stein et al., 2003). Many DNA repair proteins, normally soluble in the nucleoplasm, are reorganised in subnuclear foci after induction of DNA damage. DNA repair protein foci are formed on the sites of UVC-induced DNA lesions (Volker et al., 2001) or strand breaks (Nelms et al., 1998; El-Khamisy et al., 2003; Okano et al., 2003). Based on those observations, coordinated DNA repair in focal sites of the nucleus has been proposed. The importance of the nuclear architecture in these repair processes is highlighted by the finding that accumulation of unprocessed or truncated lamin A leads to chromatin structure alterations, defective DNA repair and increased genomic instability, characteristics of premature aging syndromes (Liu et al., 2005; Scaffidi and Misteli, 2006). No particular nuclear localisation has been assigned so far for the BER pathway. The nuclear form of hOGG1 is homogeneously distributed in the interphase nucleus and associates with condensed chromosomes during mitosis (Dantzker et al., 2002). However, the modulation of hOGG1 subnuclear distribution in response to oxidative stress remains largely unexplored.

In this study we have investigated the subnuclear distribution of the hOGG1 protein fused to the green fluorescent protein (GFP) in response to UVA irradiation. UVA is an important component of solar radiation that has been implicated in photocarcinogenesis. UVA induces the accumulation of reactive oxygen species (ROS) that may generate oxidative...
DNA damage, probably indirectly through the reaction with cellular photosensitisers (reviewed by Pfeifer et al., 2005). There is strong evidence for UVA-induced mutagenesis in mammals and yeast, the mutational spectra mainly reflecting transversions G:C to T:A that are the signature of the presence of 8-oxoG (Besaratinia et al., 2004b; Kozmin et al., 2005). We show that hOGG1, together with APE1, is recruited to foci colocalising with nuclear speckles in UVA-irradiated cells whereas it is homogeneously distributed in the nucleoplasm of untreated cells. Our results show that it is the oxidative stress, rather than the recognition of the 8-oxoG lesion that actively targets hOGG1 to nuclear speckles.

**Results**

**UVA irradiation induces OGG1 relocalisation into nuclear foci**

UVA toxic effects are predominantly mediated by the production of ROS generated by photosensitisation of endogenous sensitisers such as porphyrins. A precursor of porphyrins, δ-aminolevulinic (δ-ALA) acid, increases cell sensitivity to UVA (Duez et al., 2001). With the aim of exploring the subcellular distribution of hOGG1 in response to UVA, cells expressing a hOGG1-GFP construct were precultured with δ-ALA and UVA-irradiated at 0.2 J/cm². After a 2-hour recovery period, cells were fixed and observed under a confocal microscope. In non-irradiated (NI) cells hOGG1-GFP is homogeneously distributed in the nucleus (Fig. 1A). However, after UVA irradiation, hOGG1 relocalised to form nuclear foci (Fig. 1A). To characterise the UVA-induced hOGG1 foci, cells were washed with cytoskeleton buffer CSK containing detergent before fixation, to eliminate the nucleoplasmic soluble pool of the protein. In NI cells, all the hOGG1-GFP signal was washed out whereas the fraction of the protein relocalised to the nuclear foci in UVA-irradiated cells was resistant to CSK extraction (Fig. 1A).

We then asked whether the observed hOGG1 foci were directly associated with DNA. We reasoned that if this was the case, foci should be solubilised after treatment with DNase. Surprisingly, hOGG1 foci were still observed after DNA degradation (Fig. 1A). The same results were obtained after RNase treatment (Fig. 1A) or extraction with 2 M NaCl (data not shown). These observations suggest that UVA-induced hOGG1 foci are strongly associated with the nuclear matrix. To confirm the relocalisation of hOGG1 to the nuclear matrix after UVA irradiation, we performed a biochemical extraction protocol based on the in situ observations. The soluble protein fraction (S1) was recovered by extracting cells with CSK buffer. The resulting cell pellet (P1) was treated with DNaseI to solubilise DNA-associated proteins, and centrifuged. The pellet obtained (P2) corresponded to the nuclear matrix proteins. Using these extraction conditions, almost no hOGG1 retention was observed in the nuclear matrix from NI cells whereas the presence of the protein was clearly detected in the P2 fraction after UVA (Fig. 1B). Overexposure of the blot (Fig. 1B, bottom panel) shows that the endogenous hOGG1 protein is also associated with the nuclear matrix after UVA irradiation.

We next analysed the kinetics of formation of hOGG1 foci after cell irradiation with UVA. Cells expressing hOGG1-GFP were left to recover for different times after irradiation and processed for both microscope observations (Fig. 1C) and biochemical nuclear matrix extraction (Fig. 1D). Although no hOGG1-GFP was retained after CSK pre-extraction immediately after irradiation, after 30 minutes we observed a diffuse signal in extracted cells, which correlated with a weak band detected in the P2 fraction. After 60 minutes, hOGG1-GFP was clearly observed both in nuclear foci and in the P2 fraction. The percentage of cells presenting foci increased up to 2 hours after irradiation and correlates with the intensity of the hOGG1-GFP band in the P2 fraction.

The formation of hOGG1 foci as a response to UVA irradiation was observed in essentially all the cells of the non-synchronised population analysed. This suggests that hOGG1 foci formation is not dependent on the cell-cycle status of the cell. Because of the reported modulation of hOGG1 localisation during S phase (Luna et al., 2005), we investigated the hOGG1 relocalisation in cells actively replicating their DNA. BrdU incorporation was used to identify S-phase cells. No difference in hOGG1-GFP foci formation after UVA could be observed in those cells when compared with the rest of the population (Fig. 1E).

**hOGG1 foci are associated with nuclear speckles**

To establish whether the hOGG1-GFP foci were formed in association with a particular nuclear compartment, we performed double-labelling experiments with hOGG1-GFP and components of various subnuclear structures. By using propidium iodide for DNA staining, we found that UVA-induced hOGG1 foci were assembled in the interchromatin region and clearly excluded from heterochromatin domains (Fig. 2A). These results were confirmed by using the fusion protein hOGG1-DsRed and a cell line expressing H2B-GFP as a marker for heterochromatin (data not shown). The use of a specific antibody against the nucleolar protein B23 showed that the hOGG1-GFP foci were always excluded from the nucleoli (Fig. 2B). There are different nuclear bodies localised in the interchromatin region such as the PML, the Cajal bodies and the nuclear speckles (Spector, 2001). We therefore carried out colocalisation experiments of hOGG1-GFP with resident proteins of the different interchromatin structures. We observed a perfect colocalisation between hOGG1-GFP and the SC35 protein (Fig. 2C), a specific marker for nuclear speckles. To determine whether the concentration of hOGG1 in nuclear speckles of irradiated cells is an active process, we compared the localisation of hOGG1 with respect to the nuclear speckles before and after irradiation of the cells. Interestingly, the DNA glycosylase was completely excluded from nuclear speckles in NI cells, as shown in Fig. 2C and the cross-correlation function (CCF) between hOGG1 and SC35 (Fig. 2D). These results imply a dynamic response leading to the UVA-induced relocalisation of hOGG1-GFP to the nuclear speckles.

**hOGG1 recruitment to nuclear speckles is not dependent on the recognition of 8-oxoG by the DNA glycosylase**

To establish whether recruitment of hOGG1-GFP to nuclear speckles was induced by the formation of 8-oxoG, we measured the accumulation of 8-oxoG in DNA by using the alkaline elution technique. Surprisingly, despite the specific hOGG1 relocalisation induced by UVA, no significant increase in formamido pyrimidine glycosylase (Fpg)-sensitive sites, representing essentially 8-oxoG residues, was observed after irradiation, whereas the generation of CPDs (cyclobutane...
UVA-induced relocalisation of hOGG1

Pyrimidine dimers) was detected as T4-endoV-sensitive sites (Fig. 3A). These results suggest that hOGG1-GFP relocalisation could not be explained by a massive induction of 8-oxoG. However, we cannot exclude the fact that hOGG1 is responding to a modest accumulation of lesions, undetectable by the method we used. To establish whether the recognition of the lesion was involved in relocalisation, we analysed the redistribution of mutant forms of hOGG1 after UVA irradiation. The replacement by an alanine of phenylalanine 319 (hOGG1-F319A) results in a loss of affinity for 8-oxoG:C-containing DNA (van der Kemp et al., 2004). We also included in our experiments the hOGG1-K249Q mutant protein that can recognise the lesion but is unable to excise the oxidised base (Nash et al., 1997). Both mutant proteins were fused to GFP and transiently transfected into HeLa cells. Overexpression of the fusion proteins was verified in protein

Fig. 1. UVA irradiation induces hOGG1 relocalisation to foci associated to the nuclear matrix. (A) A HeLa cell line expressing hOGG1-GFP was UVA (UVA) or mock (NI) irradiated and allowed to recover for 2 hours. Cells were then either directly fixed (−CSK) or pre-extracted with detergent-containing buffer (+CSK) before fixation. DNA was stained with DAPI. UVA-treated cells were further treated with DNase (absence of DAPI staining indicating the complete degradation of DNA) or RNase. (B) NI and UVA-irradiated cells were separated into fractions S1 (soluble proteins), P1 (proteins bound to DNA) and P2 (nuclear matrix proteins). Samples were analysed by western blot with anti-hOGG1 antibodies, detecting the fusion protein (upper panel) and, after overexposure, the endogenous hOGG1 (bottom panel). Middle panel shows the nuclear matrix protein Sm. (C) UVA-irradiated cells were allowed to recover for the indicated times. Soluble proteins were extracted with CSK buffer before fixation and DAPI staining. (D) Kinetics of hOGG1 accumulation in the P2 fraction after UVA irradiation. (E) UVA-irradiated cells were incubated at 37°C for 2 hours, and BrdU was added to the medium for the last 45 minutes. BrdU was visualised using an anti-BrdU antibody (red). Bars, 4 μm.
extracts by western blot using anti-hOGG1 antibodies (Fig. 3B). The same extracts were tested for 8-oxoG DNA glycosylase activity. As expected, both mutants were unable to excise the 8-oxoG whereas hOGG1 activity was readily detected in hOGG1-GFP-transfected cells owing to overexpression of the active fusion protein (Fig. 3C). However, in UV A-irradiated cells, both hOGG1-F319A and hOGG1-K249Q were able to relocate to nuclear speckles as efficiently as the wild type (Fig. 3D). We conclude from these observations that hOGG1 relocation to nuclear speckles does not depend on its recognition or its repair of 8-oxoG.

Transcription blockage does not induce hOGG1 relocation to nuclear speckles

If the interaction of the protein with its substrate, 8-oxoG, is not the signal that drives hOGG1 to nuclear speckles, what is determining the hOGG1 distribution observed in UV A irradiated cells? Nuclear speckles have been largely involved in transcription and it is well established that when transcription is blocked, the speckles enlarge and round up as a result of the recruitment of transcription and splicing factors from the transcription active sites to speckles. The accumulation of CPDs after UV A irradiation (Fig. 3A) suggests that under our experimental conditions transcription might be blocked by UV A irradiation (Moné et al., 2001; Volker et al., 2001). As shown in Fig. 4A, in UV A-irradiated cells the SC35 pattern was indeed altered, with a higher percentage of round speckles and loss of their interconnections when compared with the NI ones, consistent with a UV A-induced transcription blockage. We used BrU incorporation experiments to determine the transcription level in NI and UV A cells. Although BrU incorporation in NI cells could be observed both in the nucleolus and nucleoplasmic foci, corresponding to RNA pol I and II transcription, respectively, the signal was strongly reduced in UV A-irradiated cells, confirming a transcription blockage under our irradiation conditions (Fig. 4B). We therefore asked whether the transcription blockage was the signal responsible for the relocation of hOGG1 to nuclear speckles. We treated hOGG1-GFP-expressing cells with the transcription inhibitors actinomycin D and a-amanitin. Although both treatments induced the modification of the SC35 pattern, reflecting an efficient transcription blockage, hOGG1-GFP remained completely excluded from speckles in both cases (Fig. 4C). At the doses used, actinomycin D blocks both RNA pol I and II and induces chromatin condensation. Consistently, more and larger heterochromatin regions were observed in actinomycin-D-treated cells (Fig. 4D), and hOGG1-GFP colocalised with those regions explaining the less homogeneous hOGG1 distribution pattern. This observation might be related to the previously reported colocalisation of HOGG1 with condensed chromosomes during mitosis (Dantzer et al., 2002). Treatment
UVA-induced relocalisation of hOGG1

of cells with α-amanitin, which blocks RNA Pol II elongation, did not have an effect on the subcellular distribution of hOGG1-GFP: the protein remained homogeneously distributed in the nucleoplasm and excluded from nuclear speckles as in non-treated cells (Fig. 4C).

To rule out the possibility that the UVA-induced CPDs could act as trigger for the relocalisation of hOGG1, we used UVC irradiation to induce CPDs in cellular DNA. After UVC irradiation of cells at 40 J/m², a large number of induced CPDs could be detected using antibodies against the lesion (data not shown). Moreover, the characteristic pattern of speckles reflecting transcription blockage was also observed (Fig. 4E). However, UVC irradiation did not result in the relocalisation of hOGG1-GFP to the nuclear speckles (Fig. 4E). We conclude from the above experiments that transcription blockage, whether associated or not with the accumulation of CPDs, is not the signal that targets hOGG1 to nuclear speckles.

hOGG1-GFP relocalisation to nuclear speckles is dependent on UVA-induced ROS

A known effect of UVA irradiation is the induction of an oxidative cellular stress. Oxidation of the redox-sensitive dye CH₂-DCFDA after UV A showed that irradiation results in a dramatic increase in ROS levels both in the cytoplasm and the nucleus (Fig. 5A). In addition, we observed a decrease of about 30% in the levels of reduced glutathione (GSH) after UVA irradiation (Fig. 5B), resulting in an increase in the redox ratio of oxidised/reduced glutathione, therefore confirming the oxidative stress induced by irradiation. Among the many different kinds of ROS induced in cells by UVA irradiation, H₂O₂ has been proposed to induce the persistent genomic instability observed after irradiation (Phillipson et al., 2002). hOGG1 subcellular distribution was not significantly altered by H₂O₂ treatment of cells (data not shown) (Campalans et al., 2005), suggesting that this molecule is not involved in the UVA-induced relocalisation of hOGG1-GFP to nuclear speckles. To test whether other ROS have a causal role in hOGG1-GFP relocalisation to speckles, we irradiated cells in the presence of each of two small antioxidant molecules, manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) and N-acetylcysteine (NAC). MnTBAP is a superoxide dismutase and catalase mimetic agent (Houstis et al., 2006). Incubation of cells with up to 300 μM MnTBAP could not prevent hOGG1 foci formation induced by UV A (results not shown). By contrast, when cells were incubated with NAC during irradiation and during recovery periods, recruitment of hOGG1 to nuclear speckles was completely abolished (Fig. 5C). In addition to contributing to the maintenance of glutathione levels, NAC acts as a free radical scavenger (Emonet et al., 1997; Morley et al., 2003). Addition of NAC after irradiation could not impede the relocalisation of hOGG1 (data not shown) suggesting that the oxidative burst produced during irradiation is enough to mediate hOGG1 redistribution. We conclude that the relocalisation of hOGG1 to the nuclear speckles after UVA irradiation is triggered by a burst of ROS, other than hydrogen peroxide or superoxides, induced at the time of irradiation.

The second enzyme of the BER pathway is recruited to nuclear speckles after UVA irradiation

We next asked whether other BER proteins were also recruited to nuclear speckles as a consequence of UVA irradiation. We

Fig. 3. hOGG1 relocalisation to nuclear speckles is not dependent on the recognition of the lesion 8-oxoG. (A) Fpg- and T4-endoV-sensitive sites and single-strand breaks (ssb) were measured by alkaline elution in non-irradiated (NI) and UVA-irradiated cells. Bars represent the average of at least three determinations ± s.d. (B) HeLa cells were transiently transfected with the plasmids encoding hOGG1-GFP or the mutant proteins K249Q-GFP and F319A-GFP. Protein extracts were analysed by western blotting with an anti-hOGG1 antibody. (C) The same protein extracts were assayed for hOGG1 glycosylase activity, S and P indicate the substrate and the product, respectively. (D) Transiently transfected cells were UVA-irradiated and analysed under the confocal microscope. Both mutant proteins K249Q and F319A, as well as the wild-type hOGG1 (green), were able to re-localise into foci co-localising with SC35 (red). Bars, 4 μm.
Fig. 4. Effect of transcription blockage on hOGG1 relocalisation to nuclear speckles. (A) Images corresponding to single optical sections from the middle of cells were thresholded and binarised and the percentage of round speckles calculated in NI and UVA-irradiated cells. Representative images are presented. (B) RNA synthesis was visualised by incorporation of BrU for 60 minutes in NI and UVA irradiated cells. BrU incorporation was detected with an antibody directed against BrdU (red). Arrows indicate nucleoli in the NI cells. (C) Cells were treated for 2 hours with the transcription inhibitors actinomycin-D (2 μg/ml) or α-amanitin (50 μg/ml) and immunostained with SC35 antibodies (red). hOGG1-GFP was excluded from speckles (see insets). (D) Actinomycin-D-treated cells were stained with PI-RNase (red). (E) hOGG1-GFP-expressing cells were UVC-irradiated and stained with the antibody against SC35 (red). Insets show the exclusion of hOGG1-GFP from nuclear speckles. Bar, 4 μm (B,C,E); 2 μm (D).
looked at the localisation pattern of the second enzyme of the pathway, the abasic (AP) endonuclease, APE1, fused to the YFP protein. As shown in Fig. 6A, in NI cells APE1-YFP was homogeneously distributed in the nucleoplasm and enriched in the nucleoli. However, 2 hours after irradiation, the pattern changed and a fraction of the protein associated with nuclear speckles indicated by its colocalisation with SC35 (Fig. 6A). When we compared the distribution of APE1-YFP with that of hOGG1-DsRed in irradiated cells, we observed that the AP endonuclease colocalised with the hOGG1 foci (Fig. 6B). Moreover, although the extraction protocol used failed to show a matrix-bound APE1 fraction after UV A irradiation (Fig. 6C, left panels), the use of the protein crosslinking agent Dithiobis[succinimidylpropionate] (DSP) allowed the detection of APE1 associated with the nuclear fraction in irradiated but not in control cells (Fig. 6C, right panels). Taken together, these results show that not only is the BER-initiating hOGG1 recruited to nuclear speckles, but APE1, the second enzyme of the pathway is also associated, although more weakly, with these nuclear bodies in response to irradiation.

**Discussion**

How does hOGG1 associate with nuclear speckles? It is generally assumed that DNA repair proteins associate with the sites of DNA damage and that DNA damage itself is the signal that directs those proteins to it. A damage recognition complex would be essential for the recruitment of all subsequent DNA repair proteins that will sequentially be assembled to the site of the damage (Nelms et al., 1998; Volker et al., 2001; Elkhamisy et al., 2003; Okano et al., 2003; Drouet et al., 2005).

Our initial observation that UV A radiation induces hOGG1 focus formation prompted us to explore this hypothesis for BER of oxidised bases. Several lines of evidence point to the accumulation of 8-oxoG lesions in DNA after cell irradiation with UV (Zhang et al., 1997a; Zhang et al., 1997b; Duez et al., 2001; Besaratinia et al., 2004a; Besaratinia et al., 2004b; Kozmin et al., 2005). Moreover, irradiation of cells with a UV A laser through a microscope lens was shown to induce the accumulation of 8-oxoG and subsequently of hOGG1 along
the laser pathway (Lan et al., 2004). However, the low level of 8-oxoG lesions induced by UVA irradiation under our experimental conditions and, more importantly, the fact that hOGG1 mutants unable to recognise and/or to excise the 8-oxoG lesion are still capable of forming nuclear foci strongly suggests that the recognition of 8-oxoG by the DNA glycosylase is not required for the recruitment of hOGG1 to the nuclear speckles. These organelles have been proposed to be sites of storage and assembly of transcription and mRNA splicing factors (Lamond and Spector, 2003; Handwerger and Gall, 2006). Transcription and splicing factors are recruited to nuclear speckles upon transcription blockade (Misteli, 2000). However, transcription blockade induced either by actinomycin D, α-amanitin or UVC irradiation failed to induce the recruitment of the DNA glycosylase to the speckles. These results clearly show that it is not the transcription blockade subsequent to UVA irradiation or the presence of CPDs in cellular DNA, that signals the relocalisation of hOGG1 to the speckles. Here we show that ROS mediate the recruitment of hOGG1 to the speckles after UVA irradiation. UVA can induce oxidative stress through several mechanisms. It has been proposed that the GSH efflux induced upon UVA irradiation increases the intracellular oxidative stress by increasing the ratio of GSSG/GSH, without intervention of ROS (He et al., 2003). However, it is also clear that interaction of the incoming light with cellular photosensitisers results in the production of various kinds of ROS (Jurkiewicz and Buettner, 1994). In agreement with our results that ruled out a role for H2O2 and superoxides in the relocalisation of hOGG1, photoexcitation of porphyrins, a type II-photosensitiser, by UVA leads to the generation of singlet oxygen species that can act on cellular macromolecules. Although ROS have been involved in the cytotoxic and mutagenic effects of UVA irradiation (Emonet et al., 1997; Morley et al., 2003), they are also important in other physiological situations for the induction of various signalling pathways involving JNK, FoxO, MAPK, JAK/STAT, p53 and other proteins. It is now clear that through protein–protein interactions and post-translational modifications, in particular protein phosphorylation, the retention and release of speckle-associated factors also reflects a response to environmental cues (Handwerger and Gall, 2006). The recruitment of hOGG1 to speckles could then be part of a cellular response to an increase in specific ROS levels. Most of the proteins that localise to nuclear speckles are continually shuttling between the speckles and the nucleoplasm (Misteli et al., 1997; Gorski et al., 2006). The fact that hOGG1-GFP could not be detected in nuclear speckles in non-irradiated cells suggests that this protein does not normally shuttle between these structures and the nucleoplasm, but rather that ROS produced by UVA irradiation are actively modifying the affinity of hOGG1 for these subnuclear structures. Post-translational modifications, such as protein phosphorylation, are involved in the regulation of protein recruitment to nuclear speckles (Misteli et al., 1997). Although hOGG1 lacks an RS domain, which is the most prominent feature shared by proteins relocating to nuclear speckles, post-translational modifications of hOGG1 such as phosphorylation (Dantzer et al., 2002; Hu et al., 2005), acetylation (Bhakat et al., 2006) as well as direct oxidation (Bravard et al., 2006) are candidates for mediating a response to an increase in ROS and relocating the protein. However, changes in nuclear speckle structural proteins or other nucleoplasm proteins as a response to ROS and thus leading to the recruitment of hOGG1 cannot be ruled out.

What could be the role of hOGG1 in the nuclear speckles? The presence in these organelles of proteins unrelated to mRNA processing suggests that other functions can take place in this compartment. Indeed, a proteomic analysis of interchromatin granule clusters has allowed the identification of proteins involved in other nuclear functions such as apoptosis and DNA repair (Saitoh et al., 2004). Protein degradation has also been detected in nuclear speckles (Rockel et al., 2005). If controversial results exist in the literature concerning the direct induction of 8-oxoG by UVA irradiation, there is consistent evidence concerning the accumulation of G:C to T:A transversions induced after UVA irradiation, indicating the presence of unrepairable 8-oxoG in the genome (Besaratinia et al., 2004b; Kozmin et al., 2005). An explanation of these results could be the transient inactivation or degradation of hOGG1 after UVA irradiation, which would result in inefficient repair. In some cases, inactivation of DNA repair proteins by genotoxic agents rather than direct DNA damage can be the cause of genomic instability (Jin et al., 2003; Bravard et al., 2006).

Another hypothesis is that, even if UVA irradiation does not induce a massive accumulation of 8-oxoG, hOGG1 could be recruited to the nuclear speckles in order to repair DNA. Other DNA repair proteins associate with nuclear matrix after induction of DNA damage, which would be consistent with the idea that DNA associated with the nuclear matrix is more efficiently repaired (Mullenders et al., 1988; Bode et al., 2000). Participation of nuclear organelles in DNA repair has also been suggested for PML bodies (Dellaire and Bazett-Jones, 2004; Ching et al., 2005). Proteins involved in DNA repair pathways, such as non-homologous end joining, homologous recombination and nucleotide excision repair, colocalise with PML nuclear bodies in a temporally regulated manner prior to and following DNA damage. However, the significance of these associations is unknown. PML nuclear bodies appear to represent nuclear sites where proteins and protein complexes involved in DNA repair, are assembled, anchored or post-translationally modified (Dellaire and Bazett-Jones, 2004). Analogously, nuclear speckles might be involved in post-translational modification, or regulation of enzymatic activity of DNA repair proteins. However, some of our results support the hypothesis of a direct role of the speckles in the repair of oxidised guanines. Following the induction of the lesions by treatment of the cells with an oxidative agent, the time frame for 8-oxoG repair is in the range of 2 to 4 hours (Hollenbach et al., 1999). The time scale of hOGG1 foci formation is therefore consistent with the repair kinetics of 8-oxoG in living cells. Another argument in favour of a repair role for hOGG1 in nuclear speckles is the recovery in this compartment after UVA irradiation of APE1, the second enzyme of the BER pathway. This suggests that once hOGG1 relocates to the speckles, repair is initiated and the rest of the machinery recruited (Marsin et al., 2003). An appealing possibility is that hOGG1, and more generally BER enzymes, in or around the speckles could have better access to its substrate owing to the chromatin opening associated with this nuclear organelle. It was shown that the histone H3-Lys4 methyltransferase SET1 together with the CpG-binding protein CFP1 co-localises with
nuclear speckles to regulate histone modification and cytokine methylation in euchromatin (Lee and Skalnik, 2005). Likewise, phosphatidylinositol diphosphate (PIP2) concentrates in nuclear speckles and nucleoli (Osborne et al., 2001). Nuclear PIP2 is thought to regulate chromatin remodelling and gene transcription (Mortier et al., 2005).

In summary, we have shown here that upon cellular UVA irradiation hOGG1, the enzyme responsible for the initiation of oxidised guanine repair, together with the second enzyme of the pathway, relocates to a specific nuclear organelle, the nuclear speckle. We also demonstrated that the signal triggering this redistribution of the enzyme is not the recognition of the lesion by itself, nor the blockage of transcription but the burst of ROS induced by irradiation. The characterisation of the intermediates in the signal cascade inducing the association of hOGG1 with the speckles and the components of the speckles interacting with hOGG1 should provide further elements to understand the function of this nuclear organelle in response to oxidative stress.

**Materials and Methods**

Plasmid constructions, cell culture and treatments

To generate the hOGG1 and APE1 fusions to fluorescent proteins, the open reading frames were amplified and cloned into pEGFP-N1, pEF1α-N1 and pDsRed-Monomer-N1 (Clontech). The GFP fusions for the hOGG1 mutant proteins K249Q and F319A were obtained by site-directed mutagenesis from the hOGG1-GFP construct, using the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene). HeLa cells were cultured in DMEM (Cambrex) containing 10% foetal bovine serum at 37°C in 5% CO2. For transient transfection we used the Effectene Transfection Reagent (Qiagen) according to the manufacturer’s instructions. Stable transfectants were selected in DMEM containing 800 μg/ml G418 and kept in 400 μg/ml G418.

Cells were grown on coverslips for in situ visualisation experiments and on Petri dishes for biochemical extraction. After an overnight incubation in serum-free medium, cells were incubated for 3 hours in fresh serum-free medium supplemented with 1 mM β-aminolevulinic acid (6-ALA). Immediately before irradiation, cells at about 80% confluence were washed twice with PBS and irradiated at 0.2 J/cm² with a UVA lamp at 365 nm (Bio-Sun System, Vilber Lourmat, RMX 3W) without the cover. For the transiently transfected cells, irradiations were performed 48 hours after transfection. For UVC irradiations (40 J/m²), a lamp emitting at 254 nm was used. Control cells were protected from irradiation. After irradiation, cells were allowed to recover at 37°C in DMEM for the indicated periods of time before fixation or extraction.

For transcription blockage, cells were incubated for 2 hours with 2 μg/ml actinomycin D (Sigma) or 50 μg/ml α-amanitin (Sigma) at 37°C. Transcription blockage was confirmed by BrU incorporation experiments. For antioxidant protection, cells were incubated with either 2 mM N-acetyl cysteine (NAC) or 50-300 μM BSO (buthionine sulfoximine) for 30 minutes at 4°C in 1 ml of ice-cold extraction buffer. The resulting pellets (P2), containing the nuclear matrix proteins, were resuspended in Laemmli buffer and boiled. The pellets were further incubated for 5 minutes at 4°C in 1 ml of ice-cold extraction buffer. The resulting pellets (P2), containing the nuclear matrix proteins, were resuspended in Laemmli buffer and boiled. Factions S1, P1 and P2 were analysed by western blot using antibodies against either hOGG1, APE1 (I. Hickson, Oxford University), anti-Sm (NeoMarkers) or anti-lamin A/C (Santa Cruz Biotechnology).

**Quantification of glutathione and DNA modification levels**

The alkaline elution method (Pflaum et al., 1997) was used to quantify cyclobutane pyrimidine dimers sensitive to T4 endo V and oxidative purine damages sensitive to Fpg. The levels of total and oxidised glutathione were determined as described (Bravard et al., 2006).

**BrdU and BrU incorporation**

To visualise DNA replication, cells were incubated for 45 minutes at 37°C in DMEM containing 20 μM BrdU (Sigma). Cells were washed twice in PBS, fixed in 3% paraformaldehyde for 10 minutes and permeabilised for 5 minutes at RT in PBS containing 0.5% Triton X-100. Incubation in blocking solution (PBS, 0.1% Triton X-100, 3% BSA, 1% normal goat serum) at 37°C for 1 hour was followed by a 30-minute incubation at 37°C with anti-BrdU (1/200) in DNase incubation buffer (0.5× PBS, 30 mM Tris-HCl pH 7.5, 0.3 mM MgCl2, 0.5 mM 2-mercaptoethanol, 0.5% BSA, 10 μg/ml DNase I). Labelling of RNA synthesis was performed by adding 1 mM 5-bromouridine (Sigma) to the culture medium for 60 minutes. BrU incorporation was detected with antibodies against BrdU following the same protocol as for BrUd detection.

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