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Review

DNA damage by singlet oxygen and cellular protective mechanisms

Lucymara F. Agnez-Lima [a,*], Julliane T.A. Melo [a], Acarízia E. Silva [a], Ana Helena S. Oliveira [a], Ana Rafaela S. Timoteo [a], Keronninn M. Lima-Bessa [a], Glaucia R. Martinez [b], Marisa H.G. Medeiros [c], Paolo Di Mascio [c], Rodrigo S. Galhardo [d], Carlos F.M. Menck [d]

[a] Departamento de Biologia Celular e Genética, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil
[b] Departamento de Bioquímica e Biologia Molecular, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, PR, Brazil
[c] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil
[d] Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil

A B S T R A C T

Reactive oxygen species, as singlet oxygen ($^1O_2$) and hydrogen peroxide, are continuously generated by aerobic organisms, and react actively with biomolecules. At excessive amounts, $^1O_2$ induces oxidative stress and shows carcinogenic and toxic effects due to oxidation of lipids, proteins and nucleic acids. Singlet oxygen is able to react with DNA molecule and may induce G to T transversions due to 8-oxodG generation. The nucleotide excision repair, base excision repair and mismatch repair have been implicated in the correction of DNA lesions induced by $^1O_2$ both in prokaryotic and in eukaryotic cells. $^1O_2$ is also able to induce the expression of genes involved with the cellular responses to oxidative stress, such as NF-κB, c-fos and c-jun, and genes involved with tissue damage and inflammation, as ICAM-1, interleukins 1 and 6. The studies outlined in this review reinforce the idea that $^1O_2$ is one of the more dangerous reactive oxygen species to the cells, and deserves our attention.

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Contents

1. Introduction

Molecular oxygen is intimately involved in living processes, although it has been shown to behave as a potential toxicant to all

* Corresponding author at: Departamento de Biologia Celular e Genética, CB – UFRN, Campus Universitário, Lagoa Nova, Natal, CP 1575, 59072-970 RN, Brazil. Tel.: +55 84 3211 9209; fax: +55 84 3215 3346.
    E-mail address: lfagnez@ufrnet.br (L.F. Agnez-Lima).

forms of life. This molecule may produce reactive derivatives (known as reactive oxygen species – ROS) as a result of energy absorption or electron transfer at many circumstances in the cells, including as byproduct escapes from metabolic reactions, respiration or photosynthesis, or as part of organisms' defenses against pathogens (innate immunological responses, for example) [1,2]. Singlet oxygen ($^1O_2$), the electronically excited state of molecular oxygen is one of its more reactive and toxic forms. The activation of molecular oxygen to that energetic state with anti-parallel spin (singlet state) requires overcoming of spin restriction. The first excited singlet state, $^1\Delta_g$, has two electrons with opposite spins in the same π^* orbital. The next higher excited singlet state, $^1\Sigma_g$, has one electron in each degenerated π^* orbital with opposite spins. Compared to the ground state of molecular oxygen (triplet), the $^1\Delta_g$ state is extremely reactive, and compared to the other electronically excited states, $^1\Delta_g$ state has a long half-life (of the order of 1 μs in water) to react chemically. This long half-life makes this excited molecule highly relevant in the biological environment [1,3]. Therefore, the term singlet oxygen will be used subsequently in this paper referring exclusively to molecular oxygen in the $^1\Delta_g$ state.

Singlet oxygen has a non-radical and electrophilic character. Thus, $^1O_2$ can induce oxidative reactions with organic compounds in its electron-rich moieties without the participation of free radicals. The high reactivity of $^1O_2$ with biological macromolecules makes it a potential aggressor when produced within the cell. This has been observed specially by its ability to damage guanine components and nucleic acids, with toxic and mutagenic effects [4]. However, recent evidence points to a possible role of this excited molecule in generating cell signals to modify gene expression. Several exciting reviews on the role of reactive oxygen damage to organisms have recently appeared, these include [5–8].

In this work, we present an overview of the consequences of the generation and reactivity of $^1O_2$ within the cell, giving emphasis to recent data revealing its damaging action to the genetic material, including mutagenesis. The cell responses to cope and repair these DNA lesions will also be summarized, calling attention to the existence of similar mechanisms in all living organisms. We will also review the evidence for $^1O_2$ as a mediator controlling gene responses in bacterial and eukaryotic cells.

2. Singlet oxygen in biological systems

The electronically excited molecular oxygen has been shown to naturally occur in several biological systems, such as a product of photo-oxidation of a variety of biological compounds and xenobiotics and also enzymatic reactions. In fact, the photodynamic effects mediated by light, O_2, and a photosensitizing dye ("photodynamic action") were the first example of the interaction of $^1O_2$ with biological systems [9]. Many investigators have studied the main implications of photosensitization, which have profound importance in cell biology as well as for helping the control of tumor growth, with the so-called photodynamic therapy [10–12]. Moreover, the ultraviolet light (UV of low energy, particularly UVA) also generates $^1O_2$ that may be responsible for several biological consequences of sunlight [13].

Mechanisms for the enzymatic formation of $^1O_2$ have been proposed to occur in several cases, including as part of the host immune defenses in inflammatory processes. It is believed that different cellular types, such as eosinophils, macrophages and neutrophils can generate this oxidant in response to inflammation [14,15]. Some examples of enzymatic formation of $^1O_2$ are those catalyzed by lactoperoxidase [16], dioxygenases [17], and lipoxygenases [18]. Singlet oxygen generation by reaction of heme proteins in a higher oxidation state ("compound I") with excess H_2O_2 was also reviewed by Cadenas [19].

In addition, $^1O_2$ takes part in reactions that can generate other oxidants. It has been suggested the production of some oxidants from $^1O_2$ and water in a reaction catalyzed by immunoglobulins, which includes H_2O_2 and ozone (O_3), highly reactive and cytotoxic molecules, resulting in direct inactivation of antigens or killing the pathogens [20–23], although they might also result in oxidized damage on tissues.

2.1. Reactivity of singlet oxygen

The reactions of various types of substrates with $^1O_2$ are quite well established. They include the Diels–Alder reaction of dienes to form endoperoxides ([2 + 4] cycloaddition), the "ene" reaction of alkenes to give allylic hydroperoxides, and the reaction with electron-rich alkenes without allylic hydrogens or sterically hindered to form 1,2-dioxetanes ([2 + 2] cycloaddition).

The high reactivity of this excited molecule implied in the use of quenchers as a mean to identify $^1O_2$ in biological systems. Two types of quenching process may occur: chemical quenching, in which the reaction of quencher (Q) with $^1O_2$ leads to a new product formation, and physical quenching, that leads to deactivation of $^1O_2$ with no O_2 consumption or product formation. In chemical quenching, different kinds of compounds may be used to trap products resulting from $^1O_2$. Trapping techniques are based on detection of the chemical product resulting from $^1O_2$ added to an appropriate substrate. The reaction must give a unique product or unique pattern of products, other oxidants cannot produce the same kind of products [24,25].

2.2. Chemiluminescence detection of singlet oxygen

The spontaneous decay of $^1O_2$ to the ground state leads to infrared photoemission, generating two types of chemiluminescence, which are, in fact, the basis for an important method for detection and characterization of $^1O_2$: the dimol emission [$^1O_2 + {}^1O_2 \rightarrow 2\,{}^3O_2 + h\nu$ (λ = 634 and 703 nm)] and the monomol emission [$^1O_2 \rightarrow {}^3O_2 + h\nu$ (λ = 1270 nm)]. The bimolecular transition can be monitored by means of a red sensitive, thermoelectrically cooled photo-multiplier tube connected to a discriminator, amplifier and recording system [26,27]. Dimol emission has often been used in complex systems such as enzymatic model reactions, suspensions of sub-cellular fractions and cells, and the so-called low-level chemiluminescence has been assumed as due to $^1O_2$ formation [28].

In 1979, Khan and Kasha developed a spectroscopic instrumentation capable of direct solution spectral studies of $^1O_2$ emission, using a thermoelectrically cooled lead sulfite detector [29]. The further development of a more sensitive spectrometer by Khan, based on a germanium diode photodetector, improved the capability for examination of many reactions generating $^1O_2$ [30]. The intensity of this emission is directly proportional to the concentration of $^1O_2$ [31] and provides a direct measure of the amount produced.

Nowadays, the simultaneous detection of both luminescences has been achieved. An apparatus equipped with two-photomultiplier tubes for chemiluminescence from $^1O_2$ has been developed [32]. In that study, $^1O_2$ was generated with reaction between sodium hypochlorite and hydrogen peroxide and the effects of sodium azide as an antioxidant, human serum albumin, and α-amino acids on the chemiluminescence based on the both emissions were examined.

2.3. Lifetime of singlet oxygen

An important concept when investigating the formation or the effects of $^1O_2$ is the use of deuterated solvent as a tool to

characterize the presence of $^1O_2$. It is based on the remarkable fact that the lifetime of $^1O_2$ is dependent of collisional deactivation *via* conversion of the electronic excitation energy of $^1O_2$ into vibrational energy of terminal bonds of a deactivating collision partner. This spin-forbidden and therefore relatively slow deactivation (3.1 μs lifetime in H_2O) occurs with rate constants that increase exponentially with the energy of the stretching vibration of the deactivating bond ($C–F < C–D < O–D < C–H < O–H$) leading to a pronounced solvent dependence. Then, in D_2O, the lifetime of $^1O_2$ is approximately 15–18 times longer than in water, and also longer in deuterated organic solvents. Deuterated water is, in fact, normally employed in biological and chemical experiments as a strategy to demonstrate that $^1O_2$ is involved in specific phenomena [33].

Furthermore, it is important to call attention for the dropping of $^1O_2$ lifetime in biological systems, from approximately 3 μs to around 100 ns [34,35], because of the rapid reaction of $^1O_2$ with surrounding biomolecules.

2.4. Combined use of approaches to investigate $^1O_2$ generation in different systems

The decomposition of organic hydroperoxides into peroxyl radicals is a potential source of $^1O_2$ in biological systems and a study showed that 5-(hydroperoxymethyl)uracil (5-HPMU), a thymine hydroperoxide within DNA, reacts with metal ions or HOCl, generating $^1O_2$. Spectroscopic evidence for generation of $^1O_2$ was obtained by measuring the bimolecular and monomolecular decay. Other evidences were obtained by D_2O enhancement of $^1O_2$ production and chemical trapping of $^1O_2$ with anthracene-9,10-divinylsulfonate (AVS) and detection of the specific AVS endoperoxide by HPLC/MS/MS [36].

Another study was conducted to investigate whether peroynitrate (O_2NOO) formed upon $ONOOH/ONOO^-$ decomposition could generate $^1O_2$. Measurement of monomol light emission in the near infrared region at 1270 nm and chemical trapping experiments showed that the decomposition of $ONOO^-$ or O_2NOOH at neutral to alkaline pH generates $^1O_2$ at a yield of *ca.* 1% and 2–10%, respectively [37].

2.5. Sources of singlet oxygen for chemical and biological studies

In order to better understand the role of $^1O_2$, a variety of sources for this molecule have been tested, as described below.

2.5.1. Photosensitized generation of singlet oxygen

There are two fundamental types of sensitized photooxygenation [38]. In the type I reaction, after light absorption by chromophores, the excited triplet sensitizer (specific dyes) may react directly with the substrate, while in the type II reaction, it interacts first with ground-state molecular oxygen ($^3O_2$) to produce $^1O_2$. Type I chemistry usually involves the production of free radicals or radical-ions by interaction of the triplet sensitizer with a reducing substrate. The radicals produced may undergo a wide variety of possible reactions such as insertion of oxygen or electron transfer to oxygen, electron or hydrogen abstraction from other substrates, initiation of free radical autoxidations, or back electron transfer reactions (Fig. 1). Some of these photosensitizers, such as methylene blue, promote mainly type II reactions, generating almost specifically $^1O_2$. This specificity allows the investigation of the potential roles of $^1O_2$ in cellular systems. $^1O_2$ can also be generated when photosynthetic pigments capture solar energy, transferring energy to O_2 [39,40]. Additionally, $^1O_2$ is produced from the activity of several peroxidases and other enzymatic reactions [41,42]. Different sources of $^1O_2$ are shown in Fig. 1.
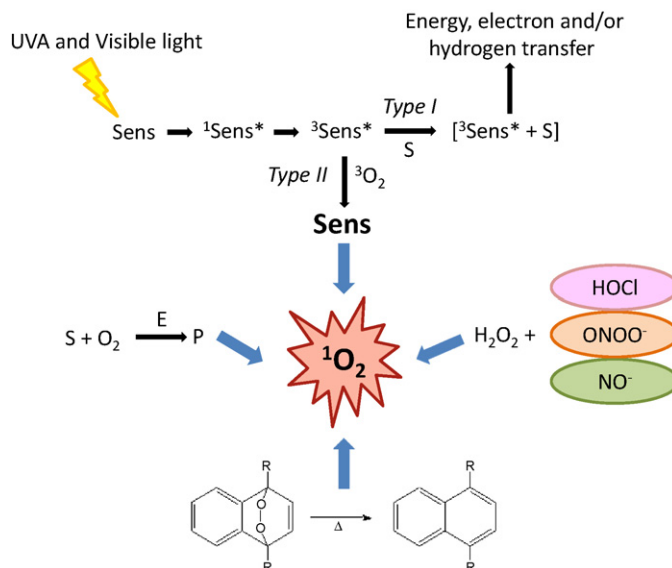

Fig. 1. Sources of singlet oxygen. On the top, type I and type II mechanisms of $^1O_2$-photosensitized generation. In the type I reaction, the excited triplet sensitizer ($^3Sens^*$) reacts directly with the substrate (S), producing a radical or radical-ion in both the sensitizer and the substrate ($[^3Sens^* + S]$). In the type II reaction, the excited triplet sensitizer transfers its excess energy to the ground-state molecular oxygen ($^3O_2$), producing $^1O_2$, which reacts with the substrate to generate oxidized products. On the left, $^1O_2$ can be also produced from the activity of several enzymes (E). On the right, chemical reactions of oxidation and disproportionation of hydrogen peroxide (H_2O_2) with hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), and nitric oxide (NO) producing $^1O_2$. Finally, on the bottom, the thermal decomposition of endoperoxide NDPO$_2$ producing $^1O_2$.

2.5.2. Oxidation and disproportionation of hydrogen peroxide

Oxidation of hydrogen peroxide by hypochlorite ion was the first indication of the presence of $^1O_2$ in a chemical system [43]. This combination must be used with caution because of the direct reaction with hydrogen peroxide or hypochlorite ion.

Disproportionation of hydrogen peroxide catalyzed by molybdate ions [44] is an interesting approach because of the high solubility of MoO_4^{2-} in water, and the huge flux of $^1O_2$ generated in aqueous solution by H_2O_2/MoO_4^{2-} system. Unfortunately, the production of $^1O_2$ using this method cannot be used in biological system due to side reactions with molybdate.

2.5.3. Thermal decomposition of endoperoxides

The thermolysis of many endoperoxides of polycyclic aromatic compounds was suggested to occur *via* the generation of excited molecular oxygen and the parent aromatic species [45]. Experimental proof for this reaction was provided using trapping technique with 9,10-diphenylanthracene-9,10-endoperoxide detection [46].

The specific production of $^1O_2$ makes these endoperoxides interesting models to understand the effects of this excited molecule in chemical, biochemical and biological systems. The hydrophilic anionic endoperoxide of disodium 1,4-naphthalenedipropanoate (NDPO$_2$) is the most used source of $^1O_2$ in biological systems [47–49]. However, more recent reports have shown that the hydrophilic non-ionic endoperoxide of N,N'-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide (DHPNO$_2$) and its parent hydrocarbon (DHPN) were able to penetrate the cells [48], adding new exciting possibilities to the use of these endoperoxides.

Using DHPN as a water-soluble naphthalene $^1O_2$ carrier and [^{18}O]-isotopically labeled molecular oxygen, the labeled endoperoxide (DHPN$^{18}O_2$) was successfully prepared [50]. This chemical source of [^{18}O]-singlet oxygen ($^{18}[^1O_2]$) allows to investigate the reactivity of $^1O_2$ toward biological targets [51], since the oxidation

Table 1
Enzymes involved on DNA damage induced by singlet oxygen.

H. sapiens	S. cerevisiae	E. coli	Substrate/function
hOGG1	yOGG1	FPG (MutM)[a]	Glycosylases for 8-oxodG:C
MYH	–	MutY	Glycosylases for 8-oxodG:A
MTH	–	MutT	8-OxodGTPase
hNTH	NTG1 (OGG2)	Endonuclease III, Endonuclease VIII[a]	Glycosylases for 8-oxodG:G or 8-oxodG:A
CSB[b], XPG, TFIIH	RAD26, RAD2 (?)	MFD (?)	Transcription-coupled repair of 8-oxodG
Other NER proteins (?)	Other NER proteins (?)	UvrABC[a]	Nucleotide excision repair
MutS homologs (?)	MSH2, MSH6	MutS	Repair of 8-oxodG:A

(?) Rationalized from the activity of the homolog from other species. There is no experimental confirmation.
  [a] Proteins with similar function, but with no homology.
  [b] CSB protein is also implicated in the repair of bulk lesions. See text for details.

products of the reactions mediated by $^{18}[^1O_2]$ will be labeled with, at least, one heavy oxygen atom, which can be detected and quantified using appropriate methods such as the high performance liquid chromatography–electrospray ionization-tandem mass spectrometry (HPLC–ESI-MS/MS) assay [50,51]. This is an important tool to identify $^1O_2$ target molecules, and the products of such reaction (Fig. 2).

3. Singlet oxygen and DNA damage

$^1O_2$ is able to react with DNA molecule, preferentially with the guanine and may form multiple products from this reaction [52]. All four bases are susceptible to oxidative damage by $^1O_2$ because of its low redox potential. However, due to the lower redox potential of guanine, this nucleobase is more susceptible to oxidation [53]. This explains the large number of injuries which may be originated from the oxidation of guanine [54]. Evidences from different laboratories identified 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) as one of the main products of the reaction of $^1O_2$ with free deoxynucleosides [55–58]. However, Niles et al. identified diastereoisomers of spiroiminodihydantoin nucleosides as the major final product formed during the methylene blue-mediated photooxidation of guanosine [59]. These results were obtained under conditions where concomitant type I photosensitization reactions may produce a bias in the interpretation and the data must be considered with caution. However, incubation of 2′-deoxyguanosine (dG) with $^1O_2$ generated by either DHPNO$_2$ or NDPO$_2$ gives rise mainly to 8-oxodG, but spiroiminodihydantoin derivatives were also detected as degradation products, confirming the results obtained with the photosensitizer [60].

Studies conducted on DNA showed that the oxidation of free monomers may differ from that in the DNA molecule. Two endoperoxides, NDPO$_2$ and DHPNO$_2$, were incubated with different concentrations of calf thymus DNA. The levels of 8-oxodG were found to increase with the concentration of these endoperoxides, and both were equally efficient in producing 8-oxodG, independently of their ionic or non-ionic character [60]. Working with plasmid DNA treated with photosensitized methylene blue or DHPNO$_2$, a potent $^1O_2$ generator, Berra et al. have observed a strong induction of base oxidized products, but few breaks or AP sites [61]. Furthermore, 8-oxodG was also found to be the main $^1O_2$ oxidation product in DNA of cells treated with DHPNO$_2$ [62]. It should be added that DNA lesions such as cyclobutane pyrimidine dimers, oxidized pyrimidine bases, 8-oxo-7,8-dihydro-2′-deoxyadenosine, and 2,6-diamino-4-hydroxy-5-formamidopyrimidine were not detected in these $^1O_2$-treated cells [62]. Additionally, it has been shown the formation of 8-oxodG as the main base oxidation product upon UVA irradiation in cellular DNA [63]. This effect is even enhanced when fibroblasts were treated with riboflavin photosensitized with UVA, since the frequency of G–T transversions, the main signature mutation of 8-oxodG, was more pronounced after treatment [64]. Several studies have shown that 8-oxodG is highly reactive toward oxidizing processes and reactive species including one-electron oxidation, peroxynitrite and $^1O_2$, respectively [59,65–68]. It was reported the qualitative identification of the predominant final $^1O_2$-oxidation products of 8-oxodG, using the HPLC–ESI-MS/MS method and the endoperoxide DHPNO$_2$ or DHPN$^{18}O_2$ acting as a clean chemical source of $^1O_2$ [60]. Thus, the 2-amino-5-[(2′-deoxy-β-D-erythro-pentofuranosyl)amino]-4H-imidazol-4-one (dIz), the 2,2-diamino-4-[(2′-deoxy-β-D-erythro-pentofuranosyl) amino]-5-(2H)-oxazolone (dOz) and the diastereomeric spiroiminodihydantoin 2′-deoxyribonucleosides (dSp) were found to be the main final stable 8-oxodG decomposition products [60]. Mass spectrometry measurements provide evidence for the formation of an oxidized nucleoside that displays the same mass spectrometric characteristics than oxidized guanidinohydantoin 2′-deoxyribonucleoside compound (dGh^{ox}). On the overall the formation of the currently reported $^1O_2$-oxidation products of 8-oxodG may be rationalized in term of the transient formation of a 5-hydroperoxide as suggested by Raoul and Cadet [67] and also proposed for the one-electron oxidation of 8-oxodG [69] (Fig. 3).

The 8-oxodG produced in a first step reaction is even easier to oxidize by $^1O_2$ than dG [70], because of its low redox potential has greater vulnerability, resulting in a higher susceptibility to further oxidation. The $^1O_2$-mediated oxidation of 8-oxodG in single stranded DNA was found to give rise to oxaluric acid as the overwhelming final stable decomposition product [71,72]. Other
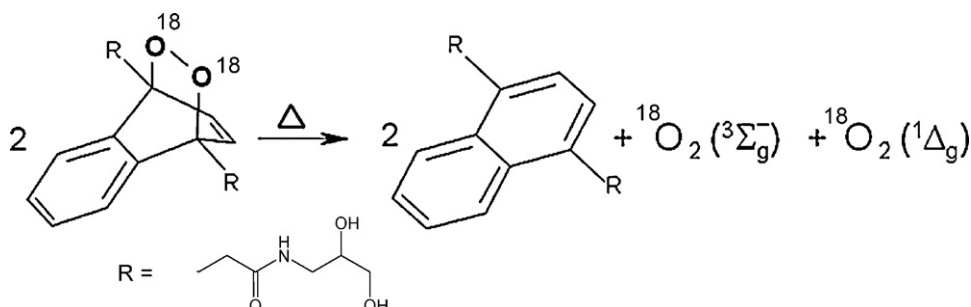

Fig. 2. Thermodissociation of hydrophilic naphthalene endoperoxide NDPO$_2$ generating singlet molecular oxygen ($^{18}O_2$ ($^1\Delta_g$)).
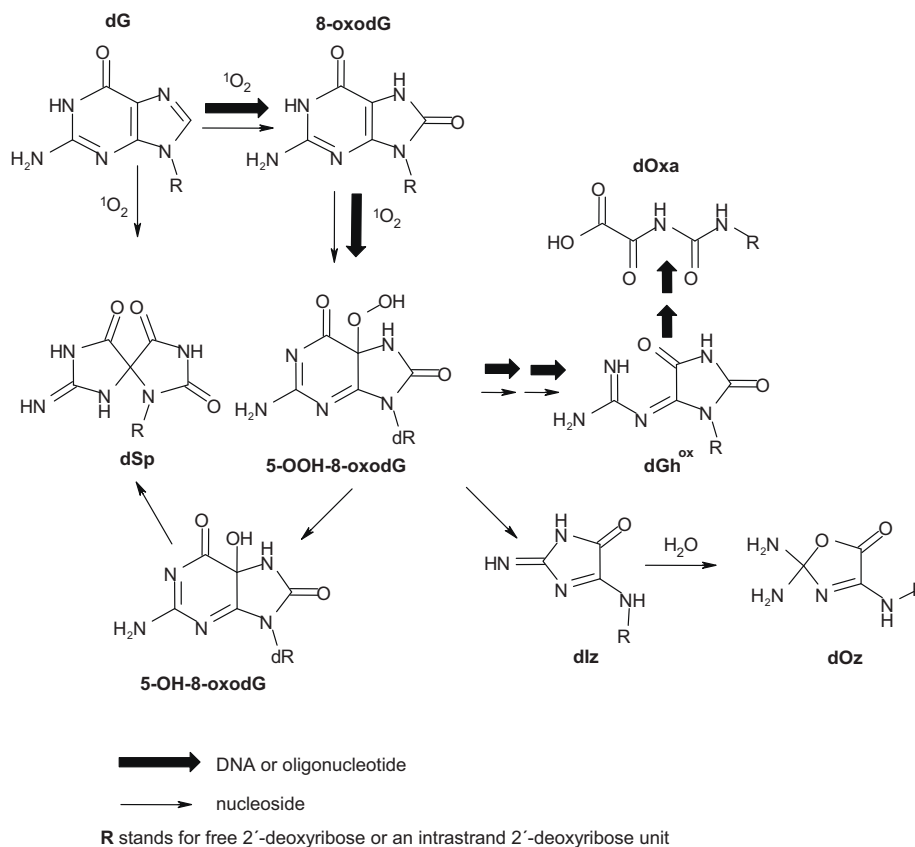
Fig. 3. Main oxidation products generated by the interaction of $^1O_2$ with the guanine moiety. Further reaction of $^1O_2$ with 8-oxodG gives rise to 5-hydroperoxide (5-OOH-8-oxodG), which decomposes to diastereomeric spiroiminodihydantoin 2′-deoxyribonucleosides (dSp), 2-amino-5-[(2-deoxy-β-D-erythro-pentofuranosyl)amino]-4H-imidazol-4-one (dIz), 2,2-diamino-4-[(2-deoxy-β-D-erythro-pentofuranosyl)amino]-5-(2H)-oxazolone (dOz), and oxidized guanidinohydantoin 2′-deoxyribonucleoside compound (dGh^ox). However, in DNA strands, it gives rise to oxaluric acid nucleoside (dOxa) through the dGh^ox intermediate.

numerous 8-oxodG oxidation products may also be produced *in vitro*, as a result from at least two sequential dG oxidation events. These products include: "guanidinohydantoin" (Gh), two diastereomers of "spiroiminodihydantoin" (Sp1 and Sp2), "imidazolone" (Iz), "oxazolone" (Oz), oxaluric acid (Oxa) and cyanuric acid (Ca) (Fig. 3) [54].

Interestingly, the nature of the $^1O_2$-mediated decomposition products of 8-oxodG strongly depends on the chemical environment of the 8-oxodG moiety [58]. Only dOxa is observed when the oxidized purine is incorporated into a short oligonucleotide; on the other hand, dOz and dSp at the exclusion of dOxa are mainly generated from $^1O_2$ oxidation of free 8-oxodG. Up to now the reasons for these differences are still unclear. Since $^1O_2$ reacts almost exclusively with guanine, the induction of these types of lesions probably occur as a consequence of the over oxidation of guanine bases, being dOz and dOxa more susceptible to hydrolysis [73]. It is important to mention that besides base modification, $^1O_2$ is also able to induce other types of DNA lesions as abasic sites (or AP sites) [74–77] and single and double strand breaks [55,78]. Moreover, single stranded DNA molecules are more reactive with $^1O_2$ than double stranded DNA, resulting in increased amounts of damage. This is probably explained by the structurally exposed position of the guanines to the oxidative agent in the single stranded DNA [79,80].

4. Biological consequences of singlet oxygen-induced DNA damage

The $^1O_2$-induced DNA damage affects directly several enzymes of the cell core metabolism. Obstruction for DNA replication was observed after the treatment of single stranded phage ⊘X174 with proflavin plus light, which was used as template for DNA polymerase I [81]. Similar results were obtained in replication *in vitro* assays testing several DNA polymerases with single stranded plasmid treated with NDPO$_2$. In addition, the analysis at sequence level indicated that the replication blockage sites correspond to the nucleotide before 2′-deoxyguanosines at the template, suggesting that the damaged guanines as the main barriers to DNA polymerases [78,82].

Reduction of bacterial transformation efficiency has been observed as consequence of phage treatment with $^1O_2$ [83]. In mammalian cells, the biological activity (measured as the ability to replicate and generate plasmid progeny) of double stranded shuttle vectors was reduced only after treatment with high doses of NDPO$_2$, whereas single stranded vectors were inactivated at much lower doses [78,84].

One of the most important consequences of $^1O_2$-induced DNA damage is mutagenesis [85]. Direct cell treatment with agents that generate $^1O_2$ is biased, as most of the damage may result from $^1O_2$ reactivity to cell components other than genetic material, such as cell membranes. Nevertheless, cell death and mutagenesis were reported in *Salmonella typhimurium* bacterial strains (including wild type and *uvrB* mutants) treated with the photosensitizer methylene blue and light [86]. Additionally, the treatment of *Escherichia coli* strains with NDPO$_2$ also induced cell death and mutagenesis, detected with the induction of rifampicin resistant colonies. Interestingly, the $^1O_2$ effects were more pronounced in strains unable to produce a functional MutY glycosylase, an enzyme responsible for the repair of dG:dA mispaired bases [87]. However, these results fail to distinguish whether these responses are due to a direct modification caused by $^1O_2$, or indirectly, by secondary products, such as products from lipoperoxidation.

A more straightforward approach (although more limited) is to perform the direct treatment of DNA molecules with $^1O_2$, in vitro, which can then be introduced into the cells, where the replication of the damaged molecules (plasmids) can promote mutations. The approaches for the mutation screening normally employ the easy and well-known bacterial genetics [88] and the sequencing of the mutations grants specific features that are expected to correspond to the fingerprinting of the mutagenic potential of $^1O_2$. Such assays have been performed in both bacteria and mammalian cells, reaching similar results. The G to T transversions are the most frequent type of mutations induced by $^1O_2$ in replicating plasmids in both prokaryotic [84,89,90] and eukaryotic cells [79,90,91]. Two other mutation types frequently observed have also been attributed to $^1O_2$ action on DNA, those are G to C transversions [91–93] and −1dG and −2dG deletions [83,94,95]. In addition, the increase of deletions was enhanced in bacteria which SOS repair was pre-induced, suggesting the involvement of this repair pathway in the generation of this type of mutation [93].

The G to T transversions have been related to 8-oxodG, which is preferentially found in the 6,8-diketo tautomeric form that in the syn conformation is able to mispair with adenine [94]. It is worth mentioning that some discrepancies in the mutagenic effects that are based on $^1O_2$-mediated oxidation of DNA templates could be due to the formation of secondary oxidation products of 8-oxodG. However, since most of the experiments performed by exposing cells to $^1O_2$ had led to observation mostly of G to T transversions, it is plausible assuming that secondary oxidations of 8-oxodG do not significantly occur in cells.

Through in vitro replication assay, Shibutani et al. observed that several DNA polymerases involved directly in the DNA replication process, such as polα, polδ and polIII, insert preferentially dAMP in front of 8-oxodG, whereas DNA polymerases involved with DNA repair synthesis, as polβ and polI, insert preferentially dCMP in front of this lesion [95]. Corroborating these data, the replication of monomodified vectors, containing a single 8-oxodG at specific sites, only resulted in G to T transversions at the lesion location, in both bacteria [96,97] and in mammalian cells [98]. Other interesting data indicate that 8-oxodGTP can also be incorporated in front of adenine during replication, leading to A to C transversions [99].

At physiological pH, 8-oxodG can also be found in the 6-enolate-8-keto tautomeric form in low frequency. In DNA, it has been shown that this molecule can adopt the anti conformation and mispairing with 2′-deoxyguanosine [94], what could explain the occurrence of G to C transversions after DNA replication. However, as stated before, in vitro replication assays, using 8-oxodG as template [95], and transfection experiments with monomodified vectors [96–98] did not show the generation of G to C transversions induced by the presence of 8-oxodG, so it is not clear what kind of lesion promotes this type of mutation in $^1O_2$-damaged DNA.

Given the abundance of 8-oxodG in cellular DNA and its easy oxidation relative to dG, it is plausible to consider the in vivo existence of lesions derived from 8-oxodG. However, the hyperoxidated lesions have not been detected in vivo, except for the Sp1 and 8-NO$_2$-G lesions [54]. The Sp1 lesion may be formed in DNA after the treatment with Cr(VI) (potassium dichromate) and excised by Nei, a base excision repair (BER) enzyme. In addition, by employing HPLC–MS analysis, Sp1 has been also detected in a nei deficient strain of E. coli, suggesting its accumulation in vivo [99], even though HPLC–MS methods have been shown to lead to overestimated values of DNA damage [100]. Interestingly, cellular mutation studies have also indicated that G–C transversions, and to a lesser degree G–T transversion mutations, predominate when Sp1 is formed in duplex DNA [99–104].

As previously discussed, the damaged base 8-oxodG is more vulnerable to oxidation by $^1O_2$ than dG, and some its oxidation products are guanidinohydantoin and oxaluric acid [72,105]. These lesions may function as templates for adenine and guanine insertion in vitro, by several DNA polymerases. Therefore, these lesions can be produced in $^1O_2$-treated DNA and may be the main cause for the G to C (and part of the G to T) transversions [106].

Wagner and Fuchs, working with monomodified vectors carrying a single 8-oxodG, and plasmids containing AP sites induced by acid treatment, investigated the induction of −1dG and −2dG deletions [93]. These authors did not observed the occurrence of −1dG and −2dG at the sites of the referred lesions, suggesting that 8-oxodG and AP sites are not responsible for the deletions induced by $^1O_2$. These data lead to the conclusion that even though 8-oxodG is the major kind of DNA damage induced by $^1O_2$, it plays an important mutagenic role only for the generation of G to T transversions. Thus, other types of DNA damage induced by this oxidative agent, even if in low frequency, may be involved in the formation of the G to C transversions and −1dG and −2dG deletions.

As expected, the sequence flanking the lesions and the DNA repair background influence both the frequency and the type of mutagenesis induced by $^1O_2$ in bacteria. Agnez-Lima et al. investigated the mutagenicity induced by $^1O_2$ in a defined sequence of DNA after replication in E. coli mutant strains with different deficiencies for DNA repair [49]. For this purpose, a plasmid containing a $^1O_2$-damaged 14 bases oligonucleotide (with only three contiguous guanosines) was introduced into the bacterial strains and mutations were screened by differential hybridization. The damaged plasmid mutated in all strains tested, but mutagenesis was significantly increased in bacteria deficient in DNA repair enzymes FPG and MutY glycosylases. In fact, the frequency of mutants in fpg mutY strain was higher than in the triple mutant fpg mutY uvrC, suggesting that the activity of the UvrABC system can favor the mutagenesis of these lesions. Additionally, the G to T transversions were observed preferentially at the first guanine (located at the 5′ side) of the target sequence, whereas the G to C transversions were preferentially induced at the third guanines. At the second guanine of the target sequence almost no mutations were observed. The G to T and G to C transversions were more frequent in fpg mutY deficient strain, suggesting the participation of both FPG and MutY glycosylase also for the repair of the DNA damage responsible for G to C mutations. These data are consistent with the idea that DNA structure at specific sequence contexts may influence the DNA repair/tolerance pathways that deal with damaged bases. Alternatively, different lesions may be produced at different positions of the target DNA.

Another interesting observation concerning $^1O_2$-induced mutagenesis is the finding that 8-oxodG can lead to mutation in the nucleotide located at 5′ of this lesion. In vitro replication of a template containing a single 8-oxodG may result in a mispair involving the cytosine before the lesion [107]. This is in agreement with the findings of Kamiya et al., who, working with monomodified vectors containing an 8-oxodG in three different positions of c-Ha-ras gene, observed mutations at the 5′ site of the 8-oxodG [108]. The authors proposed that this lesion could interfere with the double helix structure leading the DNA polymerases to insert the wrong nucleotide before the damage. Agnez-Lima et al., working with a plasmid with a $^1O_2$-damaged oligonucleotide, have also observed the occurrence of mutations at the cytosine located at 5′ of the first guanine of the target sequence [49].

Nevertheless, the majority of the results described above, which are mostly based on in vitro DNA replication of oxidatively generated DNA damage (such as 8-oxodG), may only simplify the mutagenic potential of such lesions. The whole replication

machinery involves the complex participation of several important proteins for replication processivity and fidelity, and the real mutagenic impact of these lesions may be different in these conditions. Working with yeast, van der Kemp et al. obtained evidence that the monoubiquitination of the replication clamp PCNA (proliferating cell nuclear antigen) leads to the recruitment of the translesion DNA polymerase eta, which bypass lesions such as 8-oxodG, preventing mutagenesis of this lesion [109]. In fact, *in vitro* DNA synthesis adding PCNA and RPA (replication protein A) to several DNA polymerases indicated that these proteins reduce the mutagenicity of oxidatively generated lesions [110]. More recently, the effect of 8-oxodG and abasic sites were investigated on *in vitro* synthesis by DNA polymerase epsilon (one of the replicative DNA polymerase in eukaryotes). Although this polymerase may bypass 8-oxodG with potentially misincorporation of dAMP, addition of RPA apparently prevented the extension of DNA synthesis by DNA polymerase epsilon, in these conditions. On the other hand, this polymerase has been shown to be unable to bypass abasic sites, even when PCNA and RPA were added to the reaction [111]. At least in yeast, the DNA polymerase eta is thought to be the enzyme that accurately bypass 8-oxodG, reducing the mutagenesis induced by this lesion [112]. Despite the described roles of PCNA and RPA in the mutagenesis of $^1O_2$-induced DNA damage, it is important to consider the implications of protein damage by this excited molecule. In fact, a recent work has shown that $^1O_2$, produced by photosensitization of 6-thioguanine by UVA light, specifically damage PCNA, and this effect may increase the deleterious effects of oxidative stress [113]. More recently, data obtained with yeast treated with photosensitized rufloxacin, which generates mainly $^1O_2$, revealed that deficiency in different DNA repair pathways did not lead to increased toxicity compared to the wild type strain. However, *ogg1* deficient strains showed elevated levels of mutagenesis (mostly G:C to T:A transversions), indicating that 8-oxodG is not cytotoxic, but is highly mutagenic. Moreover, in a *ogg1* deficient background, a second mutation in the *msh6* gene, deficient in mismatch repair, or in the *rad30* gene, which encodes DNA polymerase eta, lead to a synergistic increase in $^1O_2$-induced mutagenesis, confirming that both mismatch repair (see below for more details) and this translesion polymerase are involved in the repair of 8-oxodG [114].

Although most of the work on the biological features of 8-oxodG field involve its role on mutagenesis after DNA replication, its effect on the DNA transcription process may also lead to phenotypical changes. This has been shown by investigating the transcription of reporter genes, such as the luciferase gene, in cells transfected with constructions carrying 8-oxodG lesions. The data indicate that RNA polymerase II bypasses the lesion, but promotes an error-prone transcription, which may result in the translation of an abnormal protein. Thus, this type of lesion may also lead to transcriptional errors affecting the cell phenotype, even in non-replicating cells [115].

More recently, Pastoriza-Gallego et al. analyzed the effect of a unique 8-oxodG on transcription [116]. They have constructed non-replicative shuttle vectors containing a single 8-oxodG in the transcribed strand of the luciferase reporter gene. The 8-oxodG was positioned at different sequence contexts. They observed that luciferase activity decreased ~50% in $Csb^{-/-}$ or $Ogg1^{-/-}$ embryonic mouse cells transfected with these vectors. In $Csb^{-/-}/Ogg1^{-/-}$ cells, luciferase activity decreased up to 90%. These results indicate that 8-oxodG repair depends of the CSB and OGG1 protein activities.

5. DNA repair pathways implicated in the repair of $^1O_2$-induced DNA damage

Three of the main DNA repair pathways, nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR), have been implicated in the correction of DNA lesions introduced by $^1O_2$. BER is thought to play a major role for these lesions, since it is the main pathway for other DNA damage generated by oxidative mechanisms [117]. Several DNA glycosylases, proteins that catalyze the first step on this pathway, were shown to recognize a myriad of base lesions, including those generated by $^1O_2$ attack [118]. However, many results implicate NER and MMR in the prevention of the deleterious consequences of $^1O_2$-induced DNA damage. Detailed mechanistic explanations of each of these pathways may be found in the excellent reviews [119–122]. Repair of $^1O_2$-induced damage has been studied directly by the *in vitro* action of the repair endonucleases on DNA treated with a source of this agent or by assessing the biological activity of the damaged DNA from phage or plasmid [123]. Several BER enzymes have been shown to incise DNA treated with $^1O_2$ [123,124]. Using phage M13 DNA and plasmid DNA, respectively, two different groups found evidence that both the FPG glycosylase and the UvrABC system of *E. coli* are able to repair the lesions introduced by $^1O_2$, as suggested by survival and mutagenesis assays [124–127]. Furthermore, both AP endonucleases from *E. coli*, endonuclease IV and exonuclease III, may equally complete BER of $^1O_2$-induced damage after FPG excision [126]. In addition, *E. coli xthA* mutants (exonuclease III deficient) display increased mutagenesis in plasmidial $^1O_2$-treated DNA [89]. As described above, the UvrABC system (NER) seems to participate in the repair of lesions induced by $^1O_2$, although the collected data point to a mutagenic action of NER [49]. Additionally, *E. coli* UvrABC pathway was also demonstrated to be able to remove thymine glycol and abasic sites, the two major products of oxidative DNA-damage [128]. Taken all together, these results suggest that both NER and BER may operate in the prevention of the deleterious effects of $^1O_2$ in *E. coli*. As 8-oxodG is the major lesion induced by $^1O_2$, although abasic sites, DNA strand breaks and other guanine modifications are also introduced to a lesser extent, much may be deduced about the repair of $^1O_2$-induced DNA damage from extensive studies of 8-oxodG repair systems in model organisms.

In *E. coli*, the GO system seems to provide the main protection against the mutagenic effects of 8-oxodG [127]. This system consists of three proteins: FPG (MutM), MutY and MutT. The former is the glycosylase that removes 8-oxodG, when it is pairing with a cytosine, and also cleaves the resulting abasic site by sequential β and δ-elimination reactions [129,130]. MutY is also a glycosylase, which removes adenine residues mispaired with 8-oxodG or dG, thus preventing the fixation of mutations after a first round of replication after the introduction of the lesion. Zhang et al. observed that the deficiency in *mutY* gene is related to the increase of GC to CG transversions and proposed that MutY may also remove guanine from 8-oxodG:G mispairs [131], however *in vitro* and in cells assays the 8-oxodG was not involved in GC to CG transversions [95–98]. Finally, MutT is the nucleotide triphosphatase that detoxifies the nucleotide pool of the cell hydrolyzing 8-oxodGTP to 8-oxodGMP, preventing the misincorporation of the altered nucleotide in nascent DNA strands [132] (Fig. 4).

Most of the mutagenic effects of $^1O_2$-induced damage follow similar patterns in bacteria and mammalian cells. These observations are consistent with the genome data showing that many DNA repair genes are, in general, homologous among prokaryotic and eukaryotic cells, and even though when no homology can be detected, the same mechanistic pathways, with step-by-step similarities, seem to take place [133]. Thus, the living cells have evolved equivalent strategies to clean their genetic material from insult [134]. In the case of mutagenesis, $^1O_2$-induced DNA damage that is not removed by repair enzymes may result in the same type of mutations in evolutionarily distant organisms. The GO system is a good example of such conservation, as most of the bacterial components are found in human cells, although, curiously, it seems
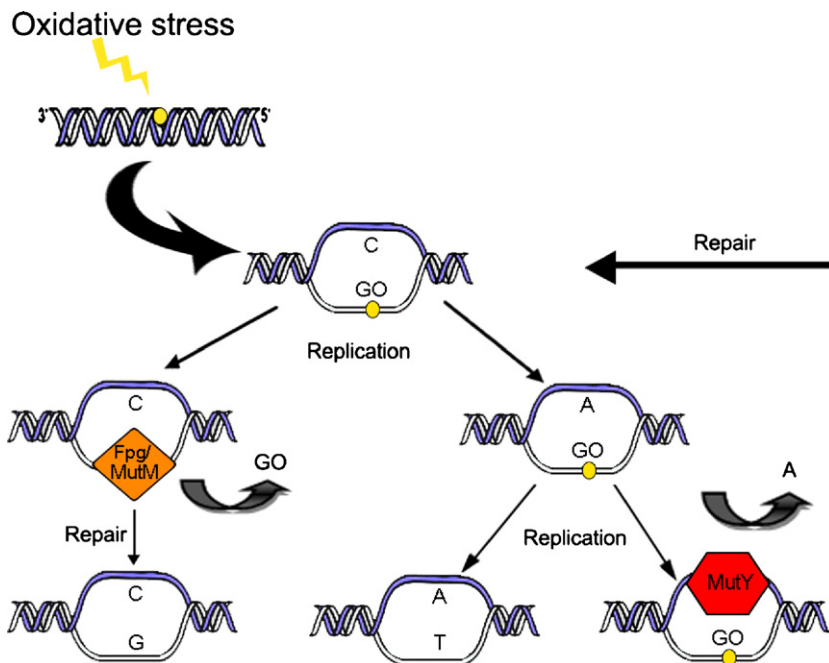
Fig. 4. The GO system and the formation of GC-TA transversions. The presence of 8-oxodG (*GO*) in DNA can cause *G* to *T* transversions if the MutM and MutY glycosylases do not act on DNA and two replication events take place. Both DNA glycosylases MutM and MutY are involved on excision of bases from DNA. After the appearance of GO in the genome, the DNA repair can follow two subpathways, either the excision of *GO* by MutM or the DNA replication. If the replication occurs, adenine (*A*) may misincorporate with GO and becomes substrate to MutY, which removes *A*. If MutY does not act on *A*, after a new replication event, the presence of *GO:A* mispairing causes *G–T* transversion, since *A* is used as template to *T* incorporation.

to be partially absent in yeast. In these organisms, the *OGG1* gene encodes an 8-oxodG glycosylase/AP lyase that is the functional counterpart of FPG [135,136]. In humans, a homolog of *Ogg1* is present [reviewed in 138] as well as homologs for the MutY [138] and MutT [139] enzymes, called MYH and MTH, respectively.

Both yeast and mammalian *OGG1* have been shown to have splice variants that are located in mitochondria [135,140]. Yeast cells exposed to $^1O_2$ are damaged extensively in the mitochondrial DNA (mtDNA) [141], and the *OGG1* deficient strain is a mitochondrial mutator [142]. Yeast cells repair mtDNA attacked by $^1O_2$ homogenously with respect to the transcriptional status, *i.e.* there is no transcription-coupled repair [143]. Likewise, mice *OGG1*$^-$/$^-$ cells are not able to repair 8-oxodG in mitochondrial DNA [144]. MYH and MTH are also localized to mitochondria [reviewed in 146], indicating that 8-oxodG is an important threat to mtDNA.

E. coli endo III and endo VIII glycosylases, as well as the human (hNTH) and yeast (NTG1 and NTG2) endo III homologs, were assumed to be involved primarily in the repair of oxidized pyrimidines. However, it is now known that these enzymes are also implicated in the repair of guanine oxidation products. A mutation in the *nei* gene, which codes for endo VIII, increases the frequency of GC to TA transversions in a *fpg mutY* null background, indicating a backup role for this enzyme in the prevention of 8-oxodG mutagenesis [146]. Furthermore, it was observed that Endo III and hNTH1 excise 8-oxodG paired with guanine or adenine [147]. This results are consistent with reports of a OGG1 independent glycosylase activity specific for 8-oxodG:dG or 8-oxodG:dA mispairs in mammalian cells [148–150]. Yeast NTG1 also excises 8-oxodG mispaired with guanine or adenine [151], although a contradictory result concerning the repair of 8-oxodG:dA mispairing has also been reported [152]. The biological significance of the activity of endo III family enzymes toward a 8-oxodG:dG pair is rather speculative, since it has been demonstrated that 8-oxodG pairs mainly with adenine and cytosine [95–98]. On the other hand, the activity toward 8-oxodG:dA may be important specially in yeast cells to prevent AT to CG mutations

that arise by incorporation of 8-oxodGTP during replication, since there is no MutT-like activity in this organism [152] (Table 1).

Two different research groups have independently obtained knockout mice lineages for the *OGG1* gene [149,150]. There is an increase in the steady-state levels of 8-oxodG and in the mutation rates in *OGG1*$^-$/$^-$ mice, but surprisingly an increase in the incidence of tumors has not been observed. This may be explained by the action of several other backup systems for the repair of 8-oxodG *in vivo*, including NER, which was shown to be active upon this lesion being generated *in vitro* [153]. Interestingly, the *OGG1* gene seems to be important for the repair of non-transcribed sequences in mammalian cells, but the repair of lesions at the transcribed strand can be accomplished by transcription coupled NER. Le Page et al. [154] analyzed the 8-oxodG-repair in murine cells in non-transcribed and transcribed sequences. For this purpose, they utilized non-replicating double-stranded plasmids containing a single 8-oxodG:C base pair and transfected the constructions in murine cells. The researchers observed that 8-oxodG repair was faster in transcribed sequences than in non-transcribed ones, indicating that this lesion is repaired beyond transcription-coupled repair (TCR). Additionally, 8-oxodG was not removed from the non-transcribed sequences whereas the 8-oxodG repair in the transcribed sequences was efficient, showing that OGG1 is required for repair of this lesion in non-transcribed sequences.

The 8-oxodG repair by TC-NER is also dependent of BRCA1 and BRCA2 proteins. In BRCA1 and BRCA2-deficient human cells, the RNA polymerase II stalls at the 8-oxodG, resulting in the impairment of the TC-NER pathway. The complementation of BRCA1-deficient cells with BRCA1 cDNA restored the repair activity, reinforcing the role of this protein in the repair of 8-oxodG lesions sitting on transcribed DNA strands [155]. More recently, however, the UVA exposure, a known inducer of $^1O_2$, of primary skin fibroblasts did not activate the Fanconi anemia/BRCA DNA repair pathway, with no indication for the formation of double strand breaks in these cells [156].

Interestingly, the repair of 8-oxodG lesions by BER has been shown to be dependent on the CSB protein [157]. However, this activity seems to be distinct from that for removal of UV photoproducts, since efficient repair of 8-oxodG lesions takes place in CS-B cells, indicating distinct activities for this protein [158]. A reduction of expression and activity of OGG1 were also observed in CS-B cells, suggesting that CSB mutations lead to deficient transcription of the OGG1 gene and thus to deficient repair of 8-oxodG in DNA [159]. Additionally, other researchers described the role of CSB in 8-oxodG repair [160–162].

In the last years, it has been shown that the expression of a mutated version of the CSA protein is able to increase the resistance to oxidative stress of cells from a CSA patient, but fails to correct their UV hypersensitivity, suggesting that some mutations in the CSA gene may interfere with the TC-NER-dependent removal of UV lesions without affecting its role in the oxidative stress response [163].

Recently, it was observed that XPC protein is also important to repair 8-oxodG since this NER factor works as a co-factor in 8-oxodG cleavage through the stimulation of OGG1-repair activity [164]. However, more experiments are necessary to determine which step of 8-oxodG repair is actually modulated by XPC.

Taken all together, these data suggest the involvement of the TC-NER in the 8-oxodG repair. However, caution should be taken when interpreting these results, since a recent article showed that efficient but error-prone transcriptional bypass of 8-oxodG by the RNA polymerase II occurred in vivo, even though this lesion could not be repaired by the TC-NER machinery in mammalian cells [115]. The existence of TC-NER for 8-oxodG in other model systems, such as yeast and bacteria, is still an open question that remains to be addressed (Table 1).

Dianov et al. [165] also analyzed the repair pathways involved with processing of 8-oxodG in DNA by mammalian cell extracts. These researchers utilized closed circular DNA constructs containing a single 8-oxodG at a defined site as substrates to determine the patch size generated after in vitro repair by mammalian cell extracts. The results showed that 8-oxodG was repaired via the single nucleotide base excision repair. However, extracts prepared from polβ-deficient mouse cells resulted in a repair of 4–5 nucleotides in 50% of the repair events, indicating the involvement of long patch repair pathway in 8-oxodG repair and that one-half of the repair of this lesion was still accomplished through replacement of only one nucleotide, suggesting the existence of an alternative polβ-independent single nucleotide repair patch pathway for processing of 8-oxodG in DNA.

The mismatch repair system (MMR), implicated in the correction of DNA replication errors, has been also shown to be involved in the repair of 8-oxodG. DeWeese et al. [166] showed that msh2$^{-/-}$ mice cells accumulate 8-oxodG and other base damage generated by oxidation, after exposure to ionizing radiation. In yeast cells, mutation in msh2 or msh6 greatly increase the frequency of GC to TA transversions in a Ogg1 background, and a high affinity binding of the Msh2–Msh6 heterodimer to both 8-oxodG:dA and 8-oxodG:dG mispairs was demonstrated [167]. In E. coli, the overexpression of MutS decreases stationary phase mutagenesis, specifically GC to TA transversions, in both wild-type and mutY backgrounds [168]. Colussi et al. [169] reported that the levels of 8-oxodG increased in Msh2-deficient cells, indicating that this protein plays an important role in the 8-oxodG repair.

About secondary lesions of 8-oxodG, it has been observed that oxazolones and oxaluric acid, which are products of further oxidation of 8-oxodG may also be formed upon $^1O_2$ attack. These lesions are also recognized and repaired by the FPG and Endo III glycosylases [71,105,170,171]. E. coli FPG, Endo III and Endo VIII are able to repair hydantoins, mutagenic derivatives of 8-oxodG

[171,172]. In eukaryotes, yeast Ntg2 also removes oxidation products of 8-oxodG [170].

6. Singlet oxygen and gene expression

The effects of $^1O_2$ within the cells is normally described as deleterious, causing damage to DNA and other macromolecules, mutagenesis and eventually cell death. However, there are several reports that indicate this excited molecule plays also a role in signaling cell responses, helping the cell to protect itself from oxidative stress. In E. coli, treatment with $^1O_2$-generated by thermodissociation of the endoperoxide NDPO$_2$, was shown to induce the activation of the reporter gene lacZ, when under the control of the soxR promoter, indicating that the regulon soxRS is induced by this treatment. The use of antioxidants did not affect the induction promoted by NDPO$_2$ treatment, consistent with the idea that $^1O_2$ was the main signaling intermediary [173]. SoxR is one of the proteins involved with the control of the SoxRS regulon, which includes many genes involved in cell protection to oxidative stress (such as sodA and the DNA repair endonuclease IV-nfo). There are also indications that $^1O_2$ also affects the expression of the OxyR regulon, also important in oxidative stress protection. In bacteria, the deletion of oxyR gene was associated to high sensitive to methylene blue plus light, while the overexpression of this gene protects against $^1O_2$ generated by this photosensitizer [174]. In Myxococcus xanthus, the synthesis of carotene is activated by photoactivation of the endogenous tetrapyrrole protoporphyrin IX, a photosensitizer that potentially generates $^1O_2$ [175]. This is probably due to an increased expression of the CarQ protein, which corresponds to an alternative sigma transcription factor, that changes the transcriptome of M. xanthus, and increases the synthesis of carotene, an efficient quencher of $^1O_2$ [175,176]. The synthesis of carotene is also increased upon exposure to exogenous photosensitizers in the eukaryotic microorganism Phaffia rhodozyma, most likely due to the generation of $^1O_2$ [177].

The most studied effects of $^1O_2$ in gene expression have been performed in the purple photosynthetic bacterium Rhodobacter sphaeroides, in responses known as photo-oxidative stress, since this is due to the light sensitization of the bacteriochlorophyll a (Bchla), which generates $^1O_2$. In this species, the $^1O_2$ stress promotes the activation of the alternate transcription sigma factor E (encoded by the gene rpoE), which changes the transcriptome of the bacteria. Controls demonstrated that other ROS did not activate the photo-oxidative stress, confirming the specificity to $^1O_2$. Several genes are members of the RpoE regulon, including the one that encodes a glutathione peroxidase-like protein, a DNA repair photolyase, phrA, and other proteins that may help to protect the cells from oxidation. The photo-oxidative stress responses are previously described in detail (for review, see references [178,179]).

The induction of signaling pathways related to the action of $^1O_2$ has also been described for mammalian cells. Sharffetter et al. showed that UVA irradiation causes an increase on mRNA levels of collagenase (MMP-1) in skin fibroblasts [180]. UVA also promoted an increase on synthesis of interleukine-6 (IL-6), which caused an induction of an autocrine signaling of collagenase (MMP-1) [181]. Furthermore, $^1O_2$ generated by NDPO$_2$ also activated the collagenase (MMP-1) in human skin fibroblasts [182]. Even though the $^1O_2$ generation and collagenase induction are thought to occur during the inflammation response and after UVA-irradiation, these observations showed a direct relation between the two phenomena.

Klotz et al. [183] reported the activation of sub-group of mitogen-activated protein kinases (MAPK), the JNK, which was activated by intracellular $^1O_2$ generated by treatment with Rose Bengal plus light. The utilization of D$_2$O, an enhancer of the $^1O_2$

lifetime, caused an increase on activation of JNK. Additionally, quenchers of $^1O_2$ inhibited the increase of JNK activity, consistent with the participation of this excited molecule signaling the activation of this kinase. Likewise, keratinocytes (HaCaT) pre-incubated with 5-aminolevulinate-P (ALA) and irradiated with red light, which generated endogenous porphyrins, showed increase in p38 and JNK activity. In addition, melanoma cells (Bro and SkMel-23) treated with ALA under the same conditions also showed an increase in JNK and p38 activity [184].

A biological consequence of JNK activation by photoactivation was showed by Chan et al. [185]. These researchers observed that after the treatment of human epidermal carcinoma cells (A431) with Rose Bengal plus light, JNK is required for induction of apoptosis. The activation of JNK was mediated by the $^1O_2$-triggered caspase activation. Interestingly, Zhuang et al. [186] showed that despite the p38-phosphorylation was produced in HL-60 cells in response to $^1O_2$ and hydrogen peroxide, p38 mitogen-activated protein kinase did not stimulate the activation of Bid in cells treated with H_2O_2. These results showed that different oxidants can initiate distinct signaling pathways in the cells, maybe as a result of the differences on chemical reactivity of the two compounds or differences on local reaction in the cells.

Among the stress proteins regulated by photodynamic effects, heme oxygenase-1 (OH-1) has been shown to be induced by UVA radiation, hydrogen peroxide and sodium arsenite in human skin fibroblasts (FEK-4) [187]. Furthermore, treatment of cells with Rose Bengal plus visible light also promoted an increase on mRNA levels of OH-1 [188].

Relating to heat shock proteins (HSPs), Gomer et al. [189] analyzed the effects of protofrin II (PH-II), mono-L-aspartyl chlorin e6 (NPe6) and etiopurpurin (SnET2) photosensitizers in murine tumor cells (RIF-1). Treatment with NPe6 and SnET2 promoted an increase on HSP-70 expression, whereas the treatment with PH-II caused a slight effect, probably due the differences on localization of photosensitizers within the cells. Singlet oxygen also mediates the activation of genes that are involved with tissue damage and inflammation. Grether-Beck et al. [190] showed that $^1O_2$ generated by UVA induced the expression of intercellular adhesion molecule 1 (ICAM-1) and the activity of AP-2 in nuclear extracts from keratinocytes. Similar results were obtained with $NDPO_2$. Wlaschek et al. [191] showed the modulation of many cytokines under UVA radiation and photodynamic therapy. Indeed, in human skin fibroblasts, the UVA radiation stimulated the production of pro-inflammatory cytokines, interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6).

Analysis relating to regulation of nuclear transcription factor by photodynamic therapy, through PH-II or NPe6 plus visible light, showed an accumulation of *c-fos* and *c-jun* mRNA in RIF-1 tumor cells. Additionally, treatment with photoprin II plus visible light and irradiation of Rose Bengal induced the activation of *c-fos* [192].

Nuclear factor kappa B (NF-κB) is other transcription factor that is activated by $^1O_2$. Ryter and Gomer showed that $^1O_2$ generated in mouse leukemia L1210 cells after PH-II-mediated photosensitization was able to induce an increase of DNA binding activity of NF-κB [193]. Following, Vile et al. studied the role of $^1O_2$ on NF-κB activation on human skin fibroblasts (FEK-4) after treatment with UVA irradiation [194]. These researchers also observed the activation of that transcription factor, indicating that NF-κB can be a general transcription factor that is activated by photo-oxidative stress.

7. Concluding remarks

In the past few decades, strong efforts were made in order to improve our knowledge on the molecular mechanisms of $^1O_2$ production and the effects of this excited molecule on cellular metabolism. There was a clear increase in the information of how and which DNA lesions are produced by $^1O_2$ and how cells handle to recognize and repair these DNA lesions. Advances in this topic revealed how different repair systems (NER, BER, MMR and translesion synthesis) help the cells to cope with the injuries caused by $^1O_2$ and other oxidatively generated DNA damage. It is well accepted now that toxic and mutagenic lesions produced by $^1O_2$ may accumulate in cell and, if not repaired, can be able to develop mutation, degenerative diseases and cancer.

Nevertheless, the results presented also clearly indicate there is still much to be done. The involvement of NER and MMR proteins in the repair of 8-oxodG and other oxidized DNA lesions are still far from clear [195]. Although the mutagenic potential of these DNA damage is well known, possibly explaining the causal and consequence relationship of $^1O_2$ and cancer, the elucidation of how oxidative stress is linked to many DNA repair human syndromes that affect development and induce premature aging and neurodegeneration is still poorly understood. Interestingly, accumulation of 8-oxodG with age was reported recently [196], even though it is difficult to correlate DNA damage generated by oxidizing agents with the aging process, as indicated by recent work with DNA repair deficient progeroid syndromes [197]. Moreover, the metabolic function of mitochondria in respiration makes these cells' organelles as critical targets to oxidative stress, including for $^1O_2$, and very little was performed to understand how this excited molecule affects the mitochondrial genome. In fact, the role of DNA damage and repair in mitochondria in the process of aging as well as neurodegeneration still remain to be clarified at molecular level [198,199].

Also, the understanding of the potential functions of $^1O_2$ on cell signaling and gene expression is still in its beginning. The fact that bacteria and mammalian cells have gene expression changes after stress induced by photosensitizers, exposure to UVA, and endoperoxides, indicates this is a universal process, kept through evolution. Therefore, the extraordinary efforts and work developed demonstrated that $^1O_2$ is able to directly oxidize DNA and promote different cellular responses, but much is still to be done, if we want to comprehend how this excited molecule affects the fate of our cells and/or monitors their metabolic processes.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

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