Review Article

SINGLET MOLECULAR OXYGEN (\(1\text{O}_2\)): A POSSIBLE EFFECTOR OF EUKARYOTIC GENE EXPRESSION

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Abstract—Biological processes involving light may have both beneficial (photosynthesis) and destructive (photosensitization) consequences. Singlet molecular oxygen, \(1\text{O}_2\), and other reactive oxygen species such as hydrogen peroxide and hydroxyl radical, arise during the interaction of light with photosensitizing chemicals in the presence of molecular oxygen. \(1\text{O}_2\) oxidizes macromolecules such as lipids, nucleic acids, and protein, depending on its intracellular site of formation; and promotes detrimental processes such as lipid peroxidation, membrane damage, and cell death. Photochemical reactive oxygen species (ROS) generating systems induce the expression of several eukaryotic genes, which include stress proteins, early response genes, matrix metalloproteinases, immunomodulatory cytokines, and adhesion molecules. These gene expression phenomena may belong to cellular defensive mechanisms, or may promote further injury. Whereas the signal transduction pathways that link site-specific oxidative damage and gene expression are poorly understood, ROS may affect signalling components in the membrane, cytosol, or nucleus, leading to changes in phospholipase, cyclooxygenase, protein kinase, protein phosphatase, and transcription factor activities. Limited evidence for \(1\text{O}_2\) involvement in gene activation phenomena consists of deuterium oxide solvent effects, inhibition by \(1\text{O}_2\)-quenchers, sensitization by porphyrins, chemical trapping methods, and comparative effects of photosensitizing dyes and thermolabile endoperoxides. The studies outlined in this review support an hypothesis that \(1\text{O}_2\) and other ROS generated during photochemical processes such as ultraviolet-A (320–380 nm) radiation exposure, or photosensitizer mediated oxidation may have dramatic effects on eukaryotic gene expression. © 1998 Elsevier Science Inc.

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INTRODUCTION

Reactive oxygen species (ROS), including singlet molecular oxygen (\(1\text{O}_2\)), can originate as a consequence of photochemical reactions between light, oxygen, and light-reactive substances called photosensitizers.\(^{1,2,3}\) The ultraviolet-A component of sunlight (UVA: 320–380 nm), produces \(1\text{O}_2\) and other ROS through the interaction of this penetrating radiation with endogenous photosensitizers in the skin.\(^4\) Thus, exposure to natural sunlight may be an important source of ROS in humans.\(^5\) In skin disorders such as porphyrias, the accumulation of photosensitizing porphyrins leads to increased ROS production and tissue damage.\(^6\) The artificial photochemical generation of \(1\text{O}_2\) and other ROS is used to destroy malignant cells or tissue, in a process called photodynamic therapy (PDT).\(^7\) PDT is a cancer treatment protocol that involves the application of a photosensitizer in combination with the targeted delivery of laser light, at a wavelength defined by the specific absorption spectrum of the chemical.\(^8–11\) Biological \(1\text{O}_2\), however, may also arise from nonphotochemical sources such as the mac-
rophase respiratory burst and membrane lipid peroxidative chain reactions. 12,13

ROS introduced into cellular or in vivo systems by photochemical mechanisms may have dramatic effects on gene regulation, 8,14–38 including the induction of distinct stress-protein gene families (reviewed in Refs. 8,14–16), such as the heat shock proteins (HSPs), the glucose-regulated proteins (GRPs), and the 32-kDa mammalian stress protein heme oxygenase-1 (HO-I). Recent studies demonstrate the activation of several “early response” genes (c-fos, c-jun, c-myc) following in vitro PDT treatments. Other examples of genes regulated by photooxidation systems are the matrix metalloproteinases, adhesion molecules, and cytokines. Attempts have been made to define the signal transduction events triggered by ROS that result in such gene activation phenomena. Indeed, protein kinases, a phosphoprotein phosphatase, and nuclear transcription factors such as NF-κB, AP-1, AP-2, have been implicated in the regulation of gene expression by ROS.

This review will outline the major aspects of 1 O2 chemistry that are relevant to biological systems. The evidence for the modulation of eukaryotic gene expression and signal transduction pathways by various photooxidation systems will be presented, with an emphasis on UVA (320–380 nm) irradiation exposure and photosensitizers used in PDT. The mechanistic tests used to implicate the possible involvement of 1 O2 in these processes will be described. Finally, the possible functional significance of these gene activation events will be discussed.

**SINGLET OXYGEN**

Singlet molecular oxygen, (1 O2) is a highly reactive form of molecular oxygen that may harm living systems by oxidizing critical organic molecules. 1 O2 is a derivative of molecular oxygen in which all valence electrons are spin paired. It differs from ground state (triplet) molecular oxygen in the reversal of the spin direction of one electron in the outermost valence shell. The electronic configuration of singlet oxygen (1Δg, 1 O2) is simplified as (π*2pγ)2, (π2pγ)0, illustrating its nonradical nature. In contrast, ground state triplet molecular oxygen (1Δg, 3 O2) exists as a biradical, (π*2pγ)1, (π2pγ)1, such that the two outermost valence electrons occupy separate orbitals with parallel spin. Triplet molecular oxygen bears a “spin restriction” against its reaction with most organic molecules. In 1 O2, this “spin restriction” is removed, allowing this species to react as an electrophilic oxidant.

Singlet oxygen exists in two forms, (1Δg), 1 O2 (22.6 kcal/mol > 3 O2), and a higher energy form (Δg*), 1 O2 (37 kcal/mol > 3 O2). The Δg*, 1 O2 decays to the Δg, 1 O2 configuration immediately upon formation, and is thus thought to be irrelevant to biological systems.47 The decay of 1Ag, 1 O2 to the ground state has a characteristic emission at 1268 nm for unimolecular decay and 634 nm for bimolecular decay resulting from the collision of two 1 O2 molecules. The detection of low level chemiluminescence from 1 O2 monomol emission by infrared spectroscopy is used as a physical proof of 1 O2 formation.

The lifetime of 1 O2 is longer in deuterium oxide (D2O) (2–7 × 10−5 s) than in water or aqueous solution (1–4 × 10−6 s). Thus, biological responses are often compared by replacing H2O in the system with D2O, as a test for 1 O2 generation. Possible isotope effects of D2O on other ROS, however, cannot be discounted. Other tests for 1 O2 involve blocking biological effects using 1 O2 quenchers of varying specificities (e.g., azide, L-histidine, and β-carotene, 1,4-diazabicyclo[2.2.2] octane, or chemical traps (e.g., 2,5-dimethylfuran, 1,3-diphenylisobenzofuran, cholesterol). The use of chemical traps and quenchers as proof of 1 O2 formation in cellular systems is complicated by the specificity of such compounds (i.e., capacity to react with other ROS or strong oxidants), or the rapid quenching of 1 O2 with competing organic molecules. Often a combination of tests will be used to imply 1 O2 formation, and this combination may include a comparative analysis of the effects of several unrelated 1 O2 sources.

From in vitro studies it is clear that 1 O2 oxidizes many organic molecules, including membrane lipid, protein, amino acids, nucleic acids, nucleotides, pyridine nucleotides, carbohydrates, and thiols. Six principal reaction mechanisms were reviewed. 1 O2 reacts by addition reactions across carbon–carbon double bonds in unsaturated olefins (Alder “ene” reaction) to form hydroperoxides, and with conjugated diene systems (Diels–Alder reaction) to form cyclic endoperoxides. 1 O2 reacts with electron rich sulphur or nitrogen containing alkenes to form 1,2 dioxetanes. 1 O2 may also react with phenolic compounds to form hydroperoxydienones, or with sulphides to form sulphoxides. Finally, 1 O2 may be quenched to 1 O2 in energy transfer reactions with compounds such as carotenoids, bilirubin, tocopherols, phenols, nickel complexes, and azide ions.

**Mechanism for photochemical generation of ROS**

The basic photodynamic reaction pathways are shown in Fig. 1. Following photon absorption at a specific wavelength, a photosensitizer (S0) in the ground state is transformed to an excited singlet state, (S1*), such that an electron in the outermost valence shell attains a higher energy level with conservation of spin direction. The
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Photosensitizers for the generation of $^1$O$_2$ in the laboratory or the clinic

Compounds capable of generating $^1$O$_2$ and other ROS upon irradiation include many natural substances such as porphyrins (hematoporphyrin and protoporphyrin IX), chlorophylls ($a$, $b$), bilirubin IX$a$, retinal (rhodopsin), quinones, and flavins (riboflavin). Many synthetic dyes are also efficient producers of singlet oxygen, including methylene blue, acridine orange, rose bengal, and eosin.

Clinical photodynamic therapy (PDT) originated with the use of hematoporphyrin, whose characteristic red fluorescence and tumor localizing properties were exploited for the visualization and diagnosis of tumors. Acetylation and reduction of hematoporphyrin produces a complex mixture called hematoporphyrin derivative (HpD) with strong photosensitizing properties. Photofrin II (PH-II), which is a partially purified form of HpD, has received approval for clinical use in the treatment of esophageal cancer. New synthetic photosensitizers for PDT, referred to as "second generation photosensitizers," have emerged for experimental PDT applications. These compounds belong to the following structural classes: phthalocyanines, bacteriochlorins, chlorins, purpurins, verdins, and benzoporphyrin derivatives. These compounds differ from PH-II by their specific subcellular localization properties and near infrared absorption maxima, which lead to more efficient in situ photoactivation.

The $^1$O$_2$ generation from photosensitizing dyes following irradiation can be measured by monitoring the formation of endoperoxides from chemical trapping agents. Illumination of immobilized rose bengal or methylene blue produced a diffusible agent that could be trapped in 2,5-dimethylfuran, and which was cytotoxic to bacteria despite a physical separation from the sensitizing compound. The inactivation of a mouse tumor by HpD-mediated PDT was shown to involve $^1$O$_2$, by trapping in 1,3-diphenylisobenzofuran. The use of chemical trapping methods to distinguish $^1$O$_2$ generation from other ROS formed during dye-mediated photooxidation in vivo has been criticized, because the traps are sensitive to oxidation by other strong oxidants.

UVA (320–380) radiation as a $^1$O$_2$ source

Solar radiation has been divided into several functional wavelength ranges, the UVC (<280 nm), which is
excluded by the earth’s atmosphere; and the UVB (280–320 nm), the UVA (320–380 nm) and near visible (380–420 nm) components, which reach the earth’s surface. The UVC and UVB regions overlap with the DNA absorption spectrum.4 UVB causes direct DNA photo-damage, which has been related to the mutagenic and tumorigenic properties of UV radiation.64 For a description of gene activation events triggered by short wave UVC and UVB radiations, readers are directed to other recent reviews.4,16,65 In contrast, the UVA region of the solar spectrum, which is not absorbed directly by DNA, generates ROS by photochemical reactions.5 Indeed, the inactivation of mammalian cells by UVA irradiation requires molecular oxygen.66 UVA causes membrane lipid peroxidation,67,68 and cell killing.69,70 These effects can be increased by D2O, which enhances 1O2 lifetime, and diminished by sodium azide, which quenches 1O2, thus implying 1O2 involvement.68,71 Furthermore, many gene activation events triggered by UVA can also be modulated by D2O and azide (Table 1). The potentiation of UVA photokilling by glutathione depletion provides further evidence for active oxygen involvement in UVA effects.72 UVA stimulates intracellular production of 1O2 and other ROS by interaction with many endogenous chromophores, though the exact species remain to be defined.4 UVA effects also involve H2O2 formation, and metal catalyzed generation of hydroxyl radical.5 Thus UVA cannot be considered a pure source of 1O2. Protoporphyrin IX (PPIX) has been proposed as an important UVA chromophore in skin. This hypothesis is supported by the fact that stimulating endogenous PPIX synthesis by δ-aminolevulinic acid treatment strongly sensitizes human skin fibroblasts (FEK-4) to UVA photokilling (C. Pourzand, R. M. Tyrrell, unpublished studies).

Other sources of biological singlet oxygen

Singlet oxygen may originate during the macrophage respiratory burst in Reactions 1–4 below. The respiratory burst oxidase (NADPH oxidase) produces superoxide anion radical, O2− (Reaction 1), which spontaneously or enzymatically dismutates to form hydrogen peroxide, H2O2 (Reaction 2). The macrophage myeloperoxidase, or related peroxidase activities, catalyze the halide driven reduction of hydrogen peroxide, to form the oxidant hypochlorous acid HOCI (Reaction 3).13 Hypochlorous acid and H2O2 react to form singlet oxygen (Reaction 4).13,73

1. NADPH + 2O2 → 2O2− + H+ + NADP+

2. 2O2− + 2H+ → H2O2 + O2

3. H2O2 + Cl− → HOCI + OH−

4. H2O2 + HOCI → 1O2 + H2O + Cl− + H+

Singlet oxygen has also been proposed as a by-product of membrane lipid peroxidation, that arises through the disproportionation of organic peroxyl radicals by Reaction 5.12

5. 2ROO− → 1O2 + RO + ROH

The iron catalysed decomposition of H2O2 (Fenton reaction), is a biological source of the oxidant hydroxyl radical (OH) (Reaction 6). When O2− provides the electron for peroxide reduction, the reaction is called the Haber–Weiss reaction (Reaction 7).74 Recent spectroscopic evidence, which demonstrates a 1268-nm emission from a mixture of O2− and H2O2, has suggested that 1O2 may be formed as the primary product of an in vitro Haber–Weiss reaction (Reaction 8).75

6. H2O2 + Fe(II) → ·OH + OH− + Fe(III)

7. O2− + H2O2 → O2 + ·OH + OH−

8. O2− + H2O2 → 1O2 (1Δg) + ·OH + OH−

While the biological significance of this observation is unclear, it implicates the formation of 1O2 in systems that generate O2−, such as xanthine oxidase.75

REGULATION OF EUKARYOTIC GENE EXPRESSION BY PHOTOCHEMICALLY GENERATED REACTIVE OXYGEN SPECIES

The evidence that photochemically generated ROS may modulate gene expression is summarized in Table 1A, with an emphasis on the specific photooxidation systems and diagnostic tests applied. The signalling intermediates that may be affected by photochemically generated ROS are summarized in Table 1B.

REGULATION OF STRESS PROTEINS BY PHOTODYNAMIC EFFECTS

Heme oxygenase-1: A case study of an oxidant-regulated gene

Heme oxygenase-1 (HO-I) represents one of the major genes induced by UVA irradiation and in vitro pho-
todynamic therapy (PDT) treatments. The expression of HO-I, a 32-kDa stress protein, is upregulated by a broad spectrum of chemical and physical agents (reviewed in Ref. 78). HO-I is a molecular marker of oxidative stress in mammalian cells, including that caused by H$_2$O$_2$, and depletion of intracellular reduced glutathione. HO-1 activity catalyzes the oxidative cleavage of heme to form the bile pigment biliverdin IX$\alpha$, with the concomitant release of carbon monoxide and heme iron.

Ultraviolet-A radiation (320–380 nm) induced the accumulation of HO-1 mRNA and protein in the primary human skin fibroblast cell line FEK-4, as a result of an increased rate of transcription. This phenomenon

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Table 1A. Regulation of Eukaryotic Gene Expression by the Photochemical Generation of Reactive Oxygen Species

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue or Cell Type</th>
<th>Source of $^1O_2$ (Photosensitizer)</th>
<th>Tests Used for $^1O_2$ Intermediacy</th>
<th>Associated Phenomena</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme oxygenase-1</td>
<td>FEK-4, human skin fibroblasts</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td>COX activation.</td>
<td>(21,76,77)</td>
</tr>
<tr>
<td></td>
<td>RIF-1, murine fibroblasts</td>
<td>PH + red light, RB + white light</td>
<td>Histidine quenching</td>
<td></td>
<td>(78,79)</td>
</tr>
<tr>
<td></td>
<td>UVA (320–380 nm)</td>
<td>NDPO$_2$</td>
<td>Azide quenching</td>
<td></td>
<td>(80–82)</td>
</tr>
<tr>
<td>Intercellular adhesion molecule-1 (ICAM-1)</td>
<td>Human skin keratinocytes</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td>AP-2 binding activity</td>
<td>(27,28)</td>
</tr>
<tr>
<td>MMP-1 (MMP2,3)</td>
<td>Human skin fibroblasts</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td>Azide quenching</td>
<td>(24,25,83,26,39,84)</td>
</tr>
<tr>
<td>HSP70</td>
<td>RIF-1</td>
<td>PH + red light, Np6 + red light</td>
<td>g.h</td>
<td>HSF binding</td>
<td>(8,17,19)</td>
</tr>
<tr>
<td></td>
<td>Bovine cells</td>
<td>PH, BpD, PC4 + red light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GRP78</td>
<td>RIF-1, M1, V-79</td>
<td>PH + red light</td>
<td>g.h</td>
<td></td>
<td>(18,19,85)</td>
</tr>
<tr>
<td>CI-100</td>
<td>FEK-4</td>
<td>UVA (320–380 nm)</td>
<td>None</td>
<td></td>
<td>(41,42)</td>
</tr>
<tr>
<td>c-fos</td>
<td>RIF-1</td>
<td>UVA (320–380 nm)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH + red light or RB + white light</td>
<td>f,g</td>
<td>Kinase activation (unspecified)</td>
<td></td>
<td>(22,23)</td>
</tr>
<tr>
<td>c-jun</td>
<td>HeLa</td>
<td>PH + red light</td>
<td>g</td>
<td></td>
<td>(22,23)</td>
</tr>
<tr>
<td>c-myc</td>
<td>HeLa</td>
<td>PH + red light</td>
<td>g</td>
<td></td>
<td>(22)</td>
</tr>
<tr>
<td>egr-1</td>
<td>RIF-1</td>
<td>PH + red light</td>
<td>g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1, IL1</td>
<td>Human skin fibroblasts</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td>11-6/MMP-1 expression</td>
<td>(30,39)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Human skin fibroblasts</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td>Azide quenching</td>
<td>(30,39)</td>
</tr>
<tr>
<td>TNF$\alpha$</td>
<td>Murine macrophages</td>
<td>PH + red light</td>
<td>g</td>
<td>AP-1 binding</td>
<td>(29)</td>
</tr>
</tbody>
</table>

Footnotes: a: Observations include both the induction of mRNA levels and protein synthesis. b: Observation includes increases in mRNA levels. c: Observation includes both mRNA expression and increase in mRNA transcriptional rate. d: Observation includes increases in bioactivity. e: Observation includes increases in protein synthesis or secretion. f: The proof of $^1O_2$ evolution from immobilized rose bengal or methylene blue has been shown using dimethylfuran trapping methods. The quantum yield of $^1O_2$ generation from rose bengal in solution has been calculated from near infrared emission or dimethylfuran trapping. The proof of $^1O_2$ evolution from hematoporphyrin derivative, has been shown by in vivo chemical trapping in 1,3 dimethylisobenzofuran and in vitro (acetoniitrile solution) by near infrared emission. h: $^1O_2$ generation from Npe6, and BpD was calculated by near infrared fluorescence emission. i: PH-II does not induce HSP70 in RIF-1 cells in vitro, but induces in vivo. j: Induction of HSP70 protein by PH-II PDT was observed in bovine smooth muscle and fibroblasts, but not in vascular endothelial cells. k: (Bose, B., and R. M. Tyrrell, unpublished studies). l: Both extracellular generation of $^1O_2$ from immobilised rose bengal and intracellular generation of $^1O_2$ from rose bengal in solution induced HIV LTR activation, but only the latter treatment induced HIV reverse transcriptase activity.
could be reproduced in many cell types of mammalian and marsupial origin. The possible involvement of $^{1}\text{O}_2$ in the UVA induction of HO-1 was demonstrated by the enhancement of the effect in deuterium oxide (D$_2$O), which prolongs $^{1}\text{O}_2$ lifetime. Sodium azide and L-histidine, two quenchers of $^{1}\text{O}_2$, attenuated the effect. In contrast, other scavengers of ROS such as mannitol and DMSO, which have no specificity for $^{1}\text{O}_2$ but high in chemical trapping in 1,3 dimethylisobenzofuran and in vitro (acetonitrile solution) by near infrared emission.

Further evidence for the $^{1}\text{O}_2$ involvement in the UVA activation of HO-1, is provided by studies using the conditions developed for $\delta$-aminolevulinic acid photodynamic therapy (ALA PDT). ALA PDT involves the stimulation of endogenous protoporphyrin IX (PPIX) synthesis using the porphyrin precursor $\delta$-aminolevulinic acid (ALA). The treatment of primary human skin fibroblasts FEK-4 with ALA stimulates the intracellular generation of $^{1}\text{O}_2$ from immobilised rose bengal and intracellular generation of $^{1}\text{O}_2$ from rose bengal in solution induced HIV LTR activation, but only the latter treatment induced HIV reverse transcriptase activity.

### Table 1B. Regulation of Eukaryotic Signal Transduction Intermediates by the Photochemical Generation of Reactive Oxygen Species

<table>
<thead>
<tr>
<th>Signalling Intermediates</th>
<th>Tissue or Cell Type</th>
<th>Source of $^{1}\text{O}_2$ or Photosensitizer</th>
<th>Tests for $^{1}\text{O}_2$ Intermediacy</th>
<th>Associated Phenomena</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB binding activity</td>
<td>FEK-4</td>
<td>UVA (320–380 nm)</td>
<td>g</td>
<td>HIV reaction$^9$, 36,37</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>L1210</td>
<td>PH + red light</td>
<td>f</td>
<td>HIV LTR$^9$ activation (87,88)</td>
<td>(33)</td>
</tr>
<tr>
<td></td>
<td>Monocytes, macrophages</td>
<td>RB + white light</td>
<td></td>
<td></td>
<td>(38)</td>
</tr>
<tr>
<td></td>
<td>Epithelial cells</td>
<td>Methylen blue, protilavin</td>
<td></td>
<td></td>
<td>(29)</td>
</tr>
<tr>
<td>AP-1 binding activity</td>
<td>Human keratinocytes.</td>
<td>UVA (320–380 nm)</td>
<td>None</td>
<td></td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>HeLa</td>
<td>PH + red light</td>
<td></td>
<td></td>
<td>(17)</td>
</tr>
<tr>
<td>AP-2 binding activity</td>
<td>Human keratinocytes.</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td></td>
<td>(89)</td>
</tr>
<tr>
<td></td>
<td>RIF-1</td>
<td>Np6 + red light</td>
<td>Azide quenching</td>
<td></td>
<td>(89)</td>
</tr>
<tr>
<td></td>
<td>Sphingomyelinase activation</td>
<td>Pc4 + red light</td>
<td>None</td>
<td>Ceramide release</td>
<td>(90)</td>
</tr>
<tr>
<td></td>
<td>Mouse L-Y R lymphoma</td>
<td>UVA (320–380 nm)</td>
<td>None</td>
<td>Arachidonic acid release</td>
<td>(91)</td>
</tr>
<tr>
<td></td>
<td>Enzymatic activity</td>
<td>Mouse L-Y R lymphoma.</td>
<td>UVA (320–380 nm)</td>
<td>None</td>
<td>(90)</td>
</tr>
<tr>
<td></td>
<td>Macrophages, RIF-1</td>
<td>Np6 + red light</td>
<td>D$_2$O solvent effect</td>
<td></td>
<td>(91)</td>
</tr>
<tr>
<td></td>
<td>Mouse keratinocytes</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td></td>
<td>(92)</td>
</tr>
<tr>
<td></td>
<td>RIF-1</td>
<td>Np6 + red light</td>
<td>Azide quenching</td>
<td></td>
<td>(93)</td>
</tr>
<tr>
<td></td>
<td>Human skin fibroblasts</td>
<td>UVA (320–380 nm)</td>
<td>D$_2$O solvent effect</td>
<td></td>
<td>(94)</td>
</tr>
<tr>
<td></td>
<td>FEK-4</td>
<td>BpD + red lightb</td>
<td>EAS trapping</td>
<td></td>
<td>(31,40)</td>
</tr>
</tbody>
</table>

$^1$ Genes: CI-100: Clone 100 phosphatase; GRP: glucose regulated protein; HSP: heat shock protein; HIV LTR: human immunodeficiency virus long terminal repeat; HO-1: heme oxygenase-1, ICAM-1: intercellular adhesion molecule-1, IL-1: interleukin 1, IL-6: interleukin-6, MMP-1: matrix metalloproteinase-1, TNFα: tumor necrosis factor alpha.


$^3$ Cell lines: FEK-4: primary human skin fibroblasts, M1: mouse tumor cells (3-methylcholanthrene induced rhabdomyosarcoma), RIF-1: mouse radiation induced fibrosarcoma, V-79: chinese hamster lung fibroblasts.


$^5$ Footnotes: a: Observations include both the induction of mRNA levels and protein synthesis. b: Observation includes increases in mRNA levels. c: observations include both mRNA expression and increase in mRNA transcriptional rate. d: observations include increases in bioactivity. e: observation includes increases in protein synthesis or secretion, f: The proof of $^{1}\text{O}_2$ evolution from immobilized rose bengal or methylene blue has been shown using dimethylfuran trapping methods. The quantum yield of $^{1}\text{O}_2$ generation from rose bengal in solution has been calculated from near infrared emission or dimethylfuran trapping. The iron chelators block the utilization of PPIX for heme synthesis.

$^6$ HO-1 activation could be induced by ALA PDT.
to 5–10 kJ/m² (Fig. 2). Maximum HO-1 mRNA responses were achieved with the ALA treatment alone, despite the fact that ALA-chelator combinations elevated PPIX to even higher levels than ALA alone. It is likely that the iron chelators have contradictory effects by increasing intracellular PPIX concentration, but at the same time, removing iron that would otherwise be available for the propagation of oxidative damage.

Both HO-1 mRNA and protein steady state levels were increased in hamster V-79 or human FEK-4 fibroblasts by in vitro PDT treatments using PH-II or HpD in combination with visible red light, and UVA had no additional effect. The nonphotosensitizing metalloporphyrins such as cobalt-protoporphyrin IX or the heme oxygenase substrate Fe-protoporphyrin IX (HEME) induce HO-1 under dark conditions. In contrast, the xanthene dye, rose bengal, which is known to be an efficient generator of 1O₂ and induced HO-I mRNA accumulation in both cell types following visible light irradiation, but not following dark incubation. The activation of HO-I by structurally dissimilar photosensitizers argues for a role of photochemically generated ROS in the response, beyond mere structural effects of the photosensitizer compounds. The failure of ZnPc photosensitization to induce HO-1 suggests that photosensitizer subcellular localization is also a critical variable in gene activation, and this is illustrated in the following section.

**Regulation of heat shock and glucose regulated stress responses by photodynamic effects**

The mammalian heat shock response is characterized by the induced synthesis of specific heat shock proteins (HSPs: M₉ 20–30, 60, 70–73, 90, 104–110 kDa) following thermal stress, in the context of impaired cellular protein synthesis. The heat shock response is a cellular adaptive mechanism against thermal protein denaturation and correlates well with acquired transient thermotolerance. Transcriptional regulation of heat shock genes following heat shock depends on the binding of regulatory factors (heat shock factors, HSF) to specific sequence elements (heat shock elements, HSE), consisting of tandem (NGAAN) nucleotide sequence arrays in the promoters of HSP genes.

The heat shock response was studied in a murine tumor model cell line (RIF-1) subjected to PDT treatments of equal cytotoxicity (20–30% clonogenic survival), using three photosensitizers (photofrin II, PH-II, mono-L-aspartyl-chlorin e6, Npe6, and tin etiopurpurin, SnET2) with different chemical structure and intracellular localization properties. Whereas all three sensitizers may target the plasma membrane, SnET2 and NPe6 localize in the lysosomes, and PH-II localizes primarily in the mitochondria. The quantum yield of 1O₂ production from Npe6 was recently calculated (ϕₐ = 0.77 in phosphate buffer). Either Npe6 or SnET2 in combination with light treatments producing equal cytotoxicity, dramatically induced in vitro HSF DNA binding activity and HSP-70 mRNA accumulation, whereas PH-II PDT had little effect on the in vitro heat shock response. Thus, subcellular localization properties of the photosensitizers that govern the site of 1O₂ generation may be a critical determinant in whether photosensitizer-mediated PDT activates the heat shock response. The oxidant, H₂O₂, activated HSF DNA binding activity but
not HSP expression in murine cells (NIH 3T3), indicating that \(^{1}O_2\) and \(H_2O_2\) may target different sites in the activation pathway.

HSP-70 mRNA expression was induced in a mouse tumor following in vivo PDT treatments with all three photosensitizers, suggesting that additional systemic factors introduce differences between the in vivo and in vitro HSP response to PDT. The induction of HSP90 and HSP70 protein synthesis was observed following the treatment of mouse M1 cells and tumors with phototherapy using benzoporphyrin derivative (mono acid ring A) (BPD), a known generator of \(^{1}O_2\). Finally, the induction of HSP-70 protein synthesis by PH-II PDT occurred in bovine smooth muscle and fibroblasts but not in cells of endothelial origin, demonstrating differential cellular HSP responses to the same PDT conditions. There are currently no reports in the literature of the modulation of HSPs by UVA, with the exception of weak HSP-70 and HSP-104 modulation by UVA radiation in human skin fibroblasts.

The glucose regulated proteins (GRPs), a distinct stress protein family, consists of members of the following molecular weight classes (Mr 75, 78–80, 94–100 kDa). The expression of the GRPs typically follows cellular stress conditions that result in the accumulation of ‘‘malfolded’’ or denatured proteins in the endoplasmic reticulum. Conditions leading to the GRPs or ‘‘malfolded protein’’ response include glucose deprivation, anoxia, or stress treatments that inhibit protein glycosylation, disturb intracellular \(Ca^{++}\) homeostasis, or alter protein thiol oxidation state. The GRPs function as chaperones, which bind and assist in the folding and subunit assembly of secreted polypeptides in the endoplasmic reticulum (ER). Following cellular stress the GRPs are believed to function in the binding and sequestration of improperly glycosylated, malfolded, or incompletely processed proteins or protein subunits in the ER, thus preventing their accumulation as insoluble aggregates.

In vitro PH-II PDT treatments (16-h PH-II incubations followed by visible red light at 630 nm) caused an increase in GRP-78 mRNA and protein synthesis in murine RIF-1 tumor cells. Isotoxic PDT treatments using the lysosomal localizing sensitizer NPe6 failed to induce the GRPs. Likewise, a short PH-II treatment (1 h), which was shown to result in the localization of the drug primarily in the plasma membrane, also did not induce GRPs under light treatment conditions of equal cytotoxicity. As observed for HSPs, these results indicate that subcellular localization properties of the photosensitizer, or its pre-irradiation incubation conditions influence the magnitude of the response, despite an equivalent phototoxicity of the treatments. PDT using aluminum phthalocyanine tetrasulfonate (Al-Pc) induced the GRPs in V-79 cells, an effect that could by synergistically enhanced by co-treatment with the \(Ca^{++}\)-ionophore nigericin. PH-II PDT induced GRP-78 mRNA accumulation in a mammary carcinoma that was transplanted in C3H/HeJ mice. Induction of GRP protein synthesis (GRP 78, 94) was also demonstrated in mouse M1 tumor cells in vitro and M1 tumors in vivo using BPD, a known \(^{1}O_2\) source. There are currently no reports in the literature of the modulation of GRPs by UVA radiation.

**PHOTOCHEMICAL ACTIVATION OF GENES INVOLVED IN INFLAMMATION AND TISSUE DAMAGE**

**Intercellular adhesion molecule 1**

UVA irradiation of skin has immunomodulatory effects that may include the expression of pro- and anti-inflammatory mediators. The intercellular adhesion molecule-1 (ICAM-1) is one such immunomodulatory molecule that may be induced by both UVA and UVB irradiation. ICAM-1, a cell surface ligand for lymphocyte function associated antigen-1 (LFA-1), mediates intercellular contact between keratinocytes and leukocytes during inflammation. The expression of ICAM-1 mRNA, and ICAM-1 protein on the cell surface was demonstrated in human skin keratinocytes following UVA treatment. The appearance of the protein could be enhanced in \(D_2O\) and diminished by sodium azide. These effects were also duplicated using a chemical source of \(^{1}O_2\), the thermal dissociation of the endoperoxide 3,3\(-(1,4\text{-naphthylidine})\) dipropionate (NDPO\(_2\)). The AP-2 consensus sequence element in the ICAM-1 gene promoter was determined by deletion analysis to be essential for the \(^{1}O_2\)-mediated activation of the gene. Either UVA or NDPO\(_2\) treatments also induced in vitro AP-2 binding activity in nuclear extracts from keratinocytes.

**Cytokine responses**

UVA radiation or PDT applied to eukaryotic cells can modulate the production of various cytokines that are multifunctional mediators of immune and inflammatory responses. UVA stimulates the production of interleukin-1 alpha (IL-1\(\alpha\)) and interleukin-1 beta (IL-1\(\beta\)) from human skin fibroblasts, and as a consequence, stimulates interleukin-6 (IL-6) expression as well. Autocrine signalling from IL-1 and IL-6 release was essential for the UVA activation of MMP-1 collagenase in human skin fibroblasts. The induction of IL-1, IL-1\(\beta\), and IL-6 mRNA levels and corresponding bioactivities following UVA treatment was modulated by \(D_2O\) and sodium azide, and duplicated by NDPO\(_2\) treatment. UVA radiation and to a lesser extent UVB caused a dose and time dependent

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*References are referenced.*
increase of the anti-inflammatory cytokine interleukin-10 (IL-10) in cultured human keratinocytes. In separate studies, the PH-II PDT treatment of human HeLa cells triggered accumulation of IL-6 mRNA and protein synthesis. This effect coincided with increased AP-1 factor binding to the distal AP-I element in the IL-6 promoter. HpD-mediated PDT was shown to induce the secretion of tumor necrosis factor alpha, TNFα, from murine macrophages in vitro.

Matrix metalloproteinases

The degradation of extracellular matrix components in skin is regulated by the matrix metalloproteinases (MMPs), which are in turn regulated by inflammatory mediators (cytokines). The protease activities of the MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs). The imbalanced overexpression of MMP-1 (collagenase) with respect to its respective TIMP-1 leads to breakdown of connective tissue, and is a possible mechanism for premature skin aging caused by sunlight exposure. UVA radiation induces (MMP-1) collagenase mRNA accumulation in human skin fibroblasts in vivo and in vitro, whereas the radiation had no effect on collagen Type I or TIMP-1 expression. Two other matrix metalloproteinases, MMP-2 and MMP-3, were induced by UVA irradiation. Levels of collagenase mRNA were also enhanced in human skin fibroblasts following exposure to chemically generated \( {\mathrm{O}}_{2} \) released from NDPO\(_2\) treatment. Collagenase expression by UVA was augmented by \( {\mathrm{D}}_{2}\)O, diminished by sodium azide, and sensitized by uroporphyrin pretreatment, implying that photochemically generated \( {\mathrm{O}}_{2} \) was important in the response.

REGULATION OF NUCLEAR TRANSCRIPTION FACTOR RESPONSES BY PHOTOCHEMICALLY GENERATED ROS

Activation of the early response genes, and the AP-1 transcription factor following photodynamic therapy

The nuclear transcription factor oncogenes (c-fos, c-jun), also called 'early response genes,' respond by rapid increases in their transcriptional rate immediately upon cellular stimulation with mitogens or growth factors. The products of these genes function as critical regulators of cellular growth and differentiation. The c-fos and c-jun oncogene products or related family members may dimerize to form the nuclear transcription factors of the AP-1 family. The AP-I factors are regulated by changes in phosphorylation and redox states. AP-1 factors activate transcription by binding to the TRE sequences (12-O-tetradecanoyl phorbol-13 acetate, TPA, responsive element) in the 5′ regulatory regions of target genes.

PDT treatments using photofrin II (PH-II) or mono-L-aspartyl chlorin e6 (NPe6) in combination with visible light, induced the rapid accumulation of c-fos and c-jun mRNA in murine RIF-1 tumor cells, which decayed to control values by 90–120 min postirradiation, respectively. The c-fos mRNA accumulation following the PDT treatments was regulated at the transcriptional level. The photosensitizer dark incubation conditions also induced a considerable c-fos and c-jun expression, which decayed with faster kinetics. Photosensitization using rose bengal, an efficient \( {\mathrm{O}}_{2} \) generator in vitro, induced the activation of c-fos following irradiation, but not after dark incubation. Several nonspecific inhibitors of protein kinases and phospholipases blocked c-fos induction following PDT treatments, and interfered with the c-fos response following phorbol ester (TPA) treatment. This study implied that membrane signalling and protein phosphorylation events were involved in the c-fos response to PDT, but did not identify the specific kinases or phospholipases involved. Induction of egr-1 and extended induction of c-myc mRNA levels following PH-II PDT occurred in RIF-1 cells. The activation of c-fos and c-jun following PH-II PDT was observed in human HeLa cells. Activation occurred within 30 min of treatment and displayed prolonged decay kinetics in comparison with the induction produced by serum stimulation or TPA treatment. Both increased transcriptional rate and increased mRNA stability accounted for the response. The induction kinetics of c-fos and c-jun differed from those reported in the mouse model, though different PH-II loading protocols were followed. It is clear that fluctuations in media serum content may induce c-fos, and such artefacts may account for a part of the induction following dark incubation of PH-II, which binds to serum proteins.

In studies from this laboratory (B. Bose and R. M. Tyrrell, unpublished), the induction of c-fos mRNA accumulation occurred following the UVA treatment of human skin fibroblasts (FEK-4), with a maximum 6 h following irradiation. Weak stimulation of AP-1 nuclear factor binding activity to synthetic AP-1 oligonucleotides, was reported following either the PH-II PDT treatment of HeLa cells, or following the UVA irradiation of human keratinocytes. The UVA activation of AP-1 binding activity was blocked by the antioxidant N-acetyl cysteine (NAC), but not by the membrane antioxidant \( \alpha \)-tocopherol. AP-1 binding has been shown to mediate the PH-II PDT induction of the interleukin-6 gene. Finally, AP-1 sites occurring in the distal enhancers of the heme oxygenase-1 gene have been implicated in the induction response of this gene following cellular treatment with the oxidant, \( {\mathrm{H}}_{2}{\mathrm{O}}_{2} \). The role of AP-I factors in the UVA or \( {\mathrm{O}}_{2} \) mediated induction of the HO-I gene have not yet been determined.
Activation of nuclear factor κB (NF-κB) by photochemically generated ROS

NF-κB is a multifunctional eukaryotic transcription factor that is important in the regulation of gene expression, including immunomodulatory cytokine and cytokine receptor genes, lymphoid specific genes, (i.e., immunoglobulin kappa light chain), and acute phase protein genes (i.e., nitric oxide synthase).\textsuperscript{124} NF-κB exists in a dormant form in the cytoplasm, typically consisting of dimers of Rel/NF-κB family members (i.e., p50: RelA) complexed to an inhibitor protein (I-κB). Following cell stimulation, the I-κB dissociates from NF-κB by a series of phosphorylation and proteolytic degradation steps, allowing the NF-κB to migrate to the nucleus where it may function as a transactivator of gene expression.\textsuperscript{125} NF-κB can be activated in lymphoid cells in response to the oxidant hydrogen peroxide, H$_2$O$_2$.\textsuperscript{126}

The administration of Photofrin-II in combination with visible red light (630 nm) to murine L1210 cells enhanced the specific binding of nuclear extract proteins to an oligonucleotide containing a single κB consensus binding site from the murine immunoglobulin κ light chain enhancer.\textsuperscript{33} UVA treatments also induced in vitro NF-κB binding activity in nuclear extracts from primary human skin fibroblasts (FEK-4).\textsuperscript{34} This phenomenon was inhibited by α-tocopherol and other membrane antioxidants, suggesting a role for membrane lipid peroxidation, and lipid-derived second messengers in the activation pathway.\textsuperscript{34}

Although there is some evidence for the activation of κB binding activity in vitro by photodynamic agents, there are currently no examples for the functional intermediacy of NF-κB in the activation of a specific cellular gene by $^{1}$O$_2$. The PH-II PDT treatment of HeLa cells failed to induce nuclear factor in vitro binding activity to an NF-κB containing sequence from the human IL-6 gene promoter.\textsuperscript{29} Furthermore, the NF-κB-binding sequence that is found in the ICAM-1 gene promoter was not necessary for the transcriptional regulation of this gene by UVA treatment, because the deletion of this NF-κB site did not affect the response.\textsuperscript{27}

However, there is evidence for a role of NF-κB in the activation of viral promoters by photodynamically generated $^{1}$O$_2$ and other ROS.\textsuperscript{56} Methylene blue photosensitization induced the reactivation of a latent HIV-1 infection, as assayed by viral reverse transcriptase activity, and induced in vitro NF-κB DNA binding activity in bandshift assays.\textsuperscript{87} Similar effects were reported for the DNA-specific photosensitizer proflavin, which causes DNA damage by direct photooxygenation of guanine nucleotides.\textsuperscript{88} The intracellular generation of $^{1}$O$_2$ by the photodynamic dye rose bengal (RB) activated both transcription from a human immunodeficiency virus long terminal repeat (HIV-1 LTR)-reporter gene fusion integrated into the genome of epithelial cells, and the reactivation of latent HIV-1 infection. The extracellular generation of $^{1}$O$_2$ from RB-coated beads, also activated the HIV-1 promoter but was ineffective at inducing HIV-1 reactivation at isotoxic doses.\textsuperscript{36,37} The evidence for $^{1}$O$_2$ generation by rose bengal or methylene blue in solution or complexed to inert supports has been documented.\textsuperscript{57,63,95,96}

**SIGNAL TRANSDUCTION EVENTS TRIGGERED BY PHOTOCHEMICALLY GENERATED ROS**

Several studies have demonstrated activation of cellular signal transduction components following PDT or UVA irradiation, although their ultimate consequences on gene regulation are incompletely understood. Membrane-associated signal transduction pathways could play a role in $^{1}$O$_2$-mediated gene activation.\textsuperscript{79} Oxidative membrane damage may release arachidonic acid from membrane phospholipids by stimulating phospholipase A2 (PLA2) activity. Free arachidonic acid is converted into bioactive pro- and anti-inflammatory mediators by lipoxigenase, which produces leukotrienes, or cyclooxygenase, which is the rate-limiting step in prostaglandin and thromboxane biosynthesis.\textsuperscript{1,127} The UVA activation of the HO-I gene was diminished by indomethecin, an inhibitor of cyclooxygenase, implying a role for arachidonic acid metabolites in the activation pathway.\textsuperscript{79} UVA treatment also stimulated arachidonic acid release from human epidermal keratinocytes\textsuperscript{90} and induced cyclooxygenase activity in human and mouse fibroblasts, leading to prostaglandin E$_2$ (PGE$_2$) production.\textsuperscript{92} Furthermore, the treatment of murine macrophages and RIF-1 tumor cells with PH-II PDT also stimulated phospholipase A2 activity, cyclooxygenase activity, and PGE$_2$ synthesis.\textsuperscript{93} Arachidonic acid metabolites were implicated in the regulation of vascular constriction following in vivo PH-II PDT treatments of rats.\textsuperscript{94,128}

A distinct lipid-derived signalling pathway involves the release of ceramide (N-acyl sphingosine) from membrane sphingomyelin, by sphingomyelinase activity. Ceramide production occurred in mouse lymphoma cells (LY-R) treated with PDT using a phthalocyanine sensitizer (Pc-4).\textsuperscript{89} The ceramide release, and both phospholipase A2-mediated arachidonic acid release and phospholipase-C-mediated inositol triphosphate release and subsequent Ca$^{2+}$ mobilization, were associated with the apoptotic response in cells treated with PDT.\textsuperscript{89,91} We have observed no ceramide release from human skin fibroblasts treated with UVA irradiation (A. Quest and R. M. Tyrrell, unpublished studies).

Further downstream from membrane-associated events, $^{1}$O$_2$ exposure may lead to the regulation of protein kinases and phosphatases. Oxidants such as H$_2$O$_2$ and other cellular stresses, such as high dose UVC and X-irradiation, activate
the mitogen-activated protein kinase (MAPK) family of proline-directed serine/threonine kinases (reviewed in Ref. 42). Among the various MAP kinases, both the stress-activated protein kinase/c-JUN NH2-terminal kinase (SAPK/JNK2) and the high osmolarity glycerol protein kinase HOG1 (p38) were activated in murine keratinocytes, immediately following PDT treatments using benzoporphyrin derivative (BPD) as the photosensitizer.31 The extracellularly regulated protein kinases (ERK1 and ERK2), however, were not stimulated by such BPD treatment. Furthermore, UVA irradiation and rose bengal photosensitization stimulate SAPK/JNK2 activity in human skin fibroblasts.32 JNK2 catalyzes the phosphorylation of the c-jun oncoprotein, leading to activation of c-jun DNA binding activity.

Ultraviolet-A radiation, as well as heat shock and hydrogen peroxide, stimulates the mRNA accumulation and activity of CI-100, which is a (tyr-thr) phosphoprotein phosphatase that specifically targets MAPK, with a specificity for ERK2, and ERK1.41,42 The consequences of this event are currently unknown.

**BIOLOGICAL SIGNIFICANCE OF GENE REGULATION FOLLOWING PHOTOOXIDATIVE STRESS**

The functional significance of the gene activation events following photooxidative stress is incompletely understood. The various stress proteins that may be induced following photooxidative stress belong to a system of cellular defense mechanisms against a larger spectrum of chemical and physical agents (i.e., heat, ionizing radiation, UVR exposure, nutrient deprivation, anoxia, xenobiotics).112 For example, the heat shock proteins (HSPs) are strongly implicated in the development of transient thermotolerance following heat stress.107 The activation of heat shock proteins by PH-II-mediated photosensitization has been correlated with the development of acquired tolerance to subsequent heat stress.17 The glucose regulated proteins (GRPs) are a cellular adaptive response against endoplasmic reticulum protein denaturation.114 GRP expression has been correlated with transient resistance to chemotherapeutic agents such as Adriamycin.129 The induction of the GRP responses by calcium ionophores has been correlated with the development of transient tolerance to subsequent PH-II PDT treatments in murine RIF-1 cells.18

A cytoprotective role for the 32-kDa protein heme oxygenase-1 following oxidative stress conditions has also been postulated.82,130 UVA treatments induce transient resistance to subsequent UVA treatments, and this effect could be blocked by heme oxygenase-1 antisense oligonucleotides.130 HO-1 degrades cellular heme, and thus changes intracellular iron distribution. The free iron released from heme by heme oxygenase activity induces the expression of ferritin, which in turn sequesters the iron in an inert oxidized form.130,131 However, because free iron is a potentially dangerous catalytic molecule, its immediate enzymatic release from heme could lead to pro-oxidant activity in the short term.132

The significance of early response gene activation following PDT treatments is less clear. Activation of AP-1 binding activity can occur in the absence of de novo protein or mRNA synthesis and depends on post-translational modification of pre-existing factors, which in some cases are prebound to DNA target sites. The activation of c-fos and c-jun transcription thus likely plays a role in AP-1 transcription factor turnover. Whereas a protective function of c-fos has recently been postulated following exposure to UVC radiation, the role of c-fos during oxidative stress remains obscure.133

It is plausible that some gene activation phenomena may occur as ‘accidents’ resulting from the perturbation of normal signal transduction processes, resulting in aberrant cell signalling and the expression of ‘unwanted’ genes. Such events may have no functional contribution to a cellular protective mechanism but, rather, may even function to amplify cellular damage. In the case of collagenase and other MMPs, expression likely causes the amplification of the initial oxidative damage leading to skin pathology or premature aging.24 Whether heme oxygenase falls into this category of deleterious enzyme activities is still not clear.

**CONCLUSIONS**

This review has illustrated that singlet molecular oxygen, 1O2, may have profound effects on intracellular cell signalling and gene expression, when introduced into eukaryotic cells by the action of phototherapeutic agents, ultraviolet-A (320–380 nm) light exposure, or thermodabile endoperoxides (Table 1). Whereas the strict oxygen dependence for biological effects mediated by UVA or PDT treatments is well established,5,10,66 these agents are not pure sources of singlet oxygen and produce other types of ROS in vivo, such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical in unknown quantities.5,134 However, it is generally agreed that the dye rose bengal is a highly efficient source of 1O2.47,57,63,95,96 Experiments involving deuterium oxide effects, chemical traps, and 1O2 quenchers are fraught with complications of nonspecificity, but when taken together, provide reasonable evidence that 1O2 is at least partly responsible for the effects (Tables 1A and 1B). Furthermore, the intracellular site of 1O2 generation strongly influences both the targets that may be susceptible to oxidation and the outcome of the gene regulation events.17,18,36 The intermediate signal transduction pathways sensitive to photochemically generated ROS, which lead to gene expression, have been partially resolved and involve membrane, cytosolic, and nuclear
transcription factor components. 

Further work in this field will no doubt link these observations into pathways which define the site of $^{1}O_{2}$ formation, the molecular targets of oxidation, the targeted genes, and the biological consequences of their expressed activities.

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Photooxidative stress and gene regulation


**ABBREVIATIONS**

AP-1—activator protein-1
ALA—d-aminolevulinic acid
ALA PDT—d-aminolevulinic acid photodynamic therapy
BPD—benzoporphyrin derivative (mono acid ring-A)
D₂O—deuterium oxide
DMO—dimethylsulphoxide
EAS—anthracene-9,10 dihydrodiethyl disulfate
ERK—extracellularly regulated kinase
GRP—glucose regulated protein
HIV-1—human immunodeficiency virus-1
H₂O₂—hydrogen peroxide
HO-1—heme oxygenase-1
HpD—hematoporphyrin derivative
HSE—heat shock element
HSF—heat shock factor
HSP—heat shock protein
IL-1—interleukin-1
IL-6—interleukin-6
ICAM-I—intercellular adhesion molecule
JNK2—c-jun amino terminal kinase-2
LTR—long terminal repeat
MAPK—mitogen activated protein kinase
MMP-1—matrix metalloproteinase-1
mRNA—messenger ribonucleic acid
NDP₀₂—3,3’-(1,4-naphthylidene) dipropionate
NF-κB—nuclear factor kappa-B
NP66—mono-L aspartyl chlorin e6 (Nippon Petrochemicals e6)
O₂—superoxide anion radical
O₃—singlet molecular oxygen
O₃—triplet molecular oxygen
PDT—photodynamic therapy
Pc-4—silicone phthalocyanine 4
PGE₂—prostaglandin E₂
PH-II—photofrin-II
PPIX—protoporphyrin IX
RB—rose bengal
RIF-I—radiation-induced fibrosarcoma
ROS—reactive oxygen species
S—photosensitizer
S₀—ground state sensitizer
S₁*-excited singlet state sensitizer
S₃*-excited triplet state photosensitizer
SAPK—stress-activated protein kinase
SnET₂—tin etiopurpurin
TIMP—tissue inhibitor of metalloproteinase
TNFα—tumor necrosis factor alpha
TPA—12-O-tetradecanoyl phorbol-13 acetate
UVA—ultraviolet-A radiation (320–380 nm)
UVR—ultraviolet radiation
ZnPC—zinc phthalocyanine