British Medical Bulletin (1993) Vol 49, No 3 pp 523–544
© The British Council 1993

Oxidative mechanisms in carcinogenesis

K Z Guyton
T W Kensler
Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland, USA

Cancer in humans and animals is a multistep disease process. In this process, a single cell can develop from an otherwise normal tissue into a malignancy that can eventually destroy the organism. The complex series of cellular and molecular changes that occur through the development of cancers can be mediated by a diversity of endogenous and environmental stimuli. Active oxygen species and other free radicals have long been known to be mutagenic; further, these agents have more recently emerged as mediators of the other phenotypic and genotypic changes that lead from mutation to neoplasia. Free radical production is ubiquitous in all respiring organisms, and is enhanced in many disease states, by carcinogen exposure, and under conditions of stress. Free radicals may therefore contribute widely to cancer development in humans. This review explores the molecular mechanisms through which free radicals can participate in the carcinogenic process.

Clinical and epidemiological findings, as well as investigations in experimental systems, have provided evidence supporting a role for free radicals in the aetiology of cancer. The free radical-scavenging vitamins C and E have been shown to protect against cancer development in animal models, and may be chemoprotective in humans.[1, 2] Other scavengers and inhibitors of free radical processes have also been demonstrated to prevent or delay the neoplastic process.[3–5] The intake of transition metals such as iron, which facilitate the production of active oxygen species, is correlated with cancer development in humans and animals;[6, 7] the carcinogenicity of other metals, including nickel and chromium, may be similarly due to their capacity to enhance oxidative

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

stress. Further, chronic inflammatory states, wherein oxidative stress dramatically increases, are also associated with the development of human malignancies.[8] Finally, many chemical carcinogens have been shown to act through free radical metabolites or processes.[9]

The cumulative evidence implicating a causative role for free radicals in the development of cancer and other diseases is strongly convincing. An appreciation of how oxidative stress functions at the molecular level is fundamental to understanding the carcinogenic capacity of this ubiquitous stress, as well as to developing appropriate prevention strategies. The following discussion focuses on the potential molecular mechanisms underlying the ability of oxidative stress to mediate carcinogenesis.

MULTISTAGE CARCINOGENESIS

The concept of multistage carcinogenesis was initially developed in rodent skin models in the 1940s[10–12] and has since been shown to apply more generally to cancers of many species and cell types. Moreover, epidemiological data suggests that the formation of human cancer involves a multistage process. The epithelium of such diverse tissues as the stomach, liver, pancreas, bladder, colon, lung, trachea, thyroid, and mammary gland, are among those in which the multistep nature of cancer has been demonstrated experimentally. While the endpoints produced at each stage have been described and operationally defined, the overall sequence of molecular events leading to cancer, including the mutation and deregulation of both oncogenes and tumour suppressor genes, is only beginning to emerge.

Experimental carcinogenesis proceeds through at least 3 distinct stages. In **initiation**, a single, somatic cell undergoes non-lethal, heritable mutation. A mutation in the cellular machinery controlling growth or differentiation is an example of the type of genetic change that occurs in initiation. This initiating mutation may provide a growth advantage during the second stage, **promotion**. In contrast to its normal counterparts, the initiated cell can escape from cellular control mechanisms when responding to external or intracellular signals. Exposure to a tumour promoter will evoke an altered response pattern wherein initiated cells, but not the normal population, are stimulated to grow.[13] The signal to expand clonally can be provided either by direct stimulation of the initiated cell, or as an indirect result of the effects of the tumour promoter on the adjacent normal cells. Tumour promotion produces relatively benign growths that can be converted into cancer in third stage, **malignant conversion**. Like initiation, conversion requires

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

genetic alteration in which cellular growth is further deregulated and thus proceeds uncontrolled.

Oxidative stress has many diverse cellular effects. As depicted in Figure 1, this ubiquitous stress can cause mutagenicity, cytotoxicity, and stimulate changes in gene expression.[14] Furthermore, these effects are likely to interplay in the development of carcinogenesis by oxidants. Mutations induced by oxidants may initiate carcinogenesis; oxidative modification of the genetic material may also participate in the progression of benign to malignant neoplasms. Alteration of the pattern of gene expression by oxidants may function in the stimulation of the initiated cell during tumour promotion. Further, oxidant-induced toxicity in the normal population may facilitate the clonal expansion of the more resistant initiated cell during promotion.

Possible mechanisms through which oxidative stress mediates each of these processes will be discussed in detail. First, oxidant-induced mutations that can initiate carcinogenesis will be addressed. The role of the hydroxyl radical and copper ions in producing 8-hydroxy-guanine (8-hydroxy-G) and the function of this lesion in mutation, will be highlighted. A discussion of the role of free radicals in tumour promotion will follow, focusing on cellular mechanisms for reprogramming of gene expression utilized by oxidants. Finally, the ability of free radicals to mediate malignant conversion will be briefly reviewed.

HYDROXYL RADICALS MAY MEDIATE OXIDATIVE DNA DAMAGE

The mutagenicity of oxygen in bacteria was first demonstrated over 30 years ago.[15] Hyperoxia, agents that generate active oxygen species (including chemicals and radiation), or oxidants elaborated by activated neutrophils have since been shown to cause DNA damage and mutagenesis in human and animal cells.[16–20] Further, active oxygen species can cause malignant transformation in cultured cells.[21] Fenn and coworkers[15] hypothesized in 1957 that the observed mutagenicity of oxygen was '...due presumably to chromosome damage resulting from an increased concentration of free radicals'. It is now widely held that the mutagenic capacity of oxygen is due to the direct interaction of hydroxyl radicals with DNA.

Hydroxyl radicals have been detected with electron paramagnetic resonance spectroscopy under conditions of active oxygen-induced DNA damage.[22] Much evidence indicates that DNA-damaging hydroxyl radicals are produced through the interaction of hydrogen peroxide and superoxide with transition metals. For example, hydrogen peroxide and superoxide do not directly interact with DNA to produce oxidative le-

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

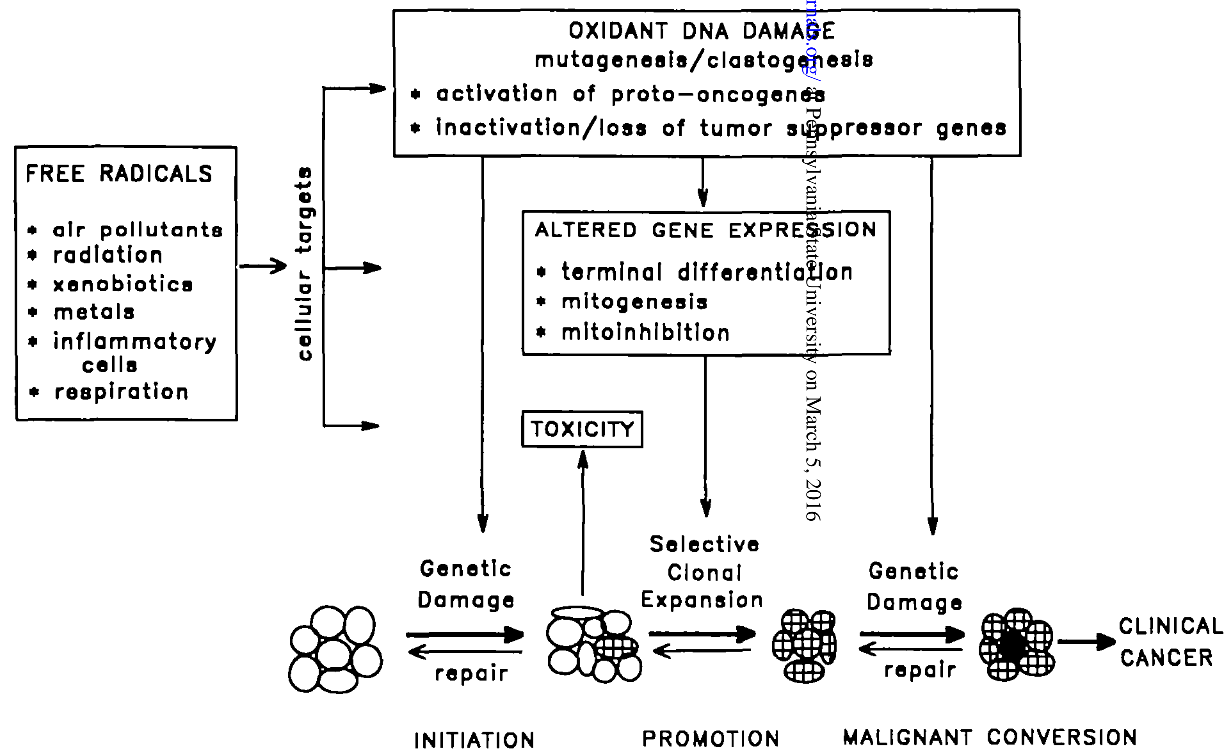

Fig. 1 Role of oxidants in multistage carcinogenesis (adapted from Reference 14).

mb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

sions in vitro (reviewed in Reference 23). Additionally, metal chelators that block the elaboration of hydroxyl radicals can inhibit DNA damage, mutations, and malignant transformation induced by active oxygen species in cell-free and in cellular systems, as can agents that detoxify hydroxyl radical or its precursors.[24–27] The carcinogenic iron complex nitriloacetic acid facilitates hydroxyl radical production from oxidants, and can enhance oxidative DNA damage; further, both iron and copper ions can also promote DNA damage by active oxygen species in vitro.[28] An unstable radical like the hydroxyl radical will interact indiscriminately with all components of the DNA molecule, producing a broad spectrum of DNA damage. Indeed, the forms of DNA damage produced by active oxygen species in experimental systems include modification of all bases as well as the production of base-free sites, deletions, frameshifts, strand breaks, DNA-protein crosslinks, and chromosomal rearrangements (reviewed in Reference 29). While the direct interaction of hydroxyl radical with the DNA molecule is implicated in the generation of many of these genetic lesions, other cellular processes, which may also be mediated through hydroxyl radical production, can contribute to oxidative DNA damage. In particular, endonucleases activated through an oxidant-stimulated rise in intracellular calcium can stimulate the formation of strand breaks and degradation products of the DNA molecule.[30]

ROLE OF OXIDATIVE DNA DAMAGE IN INITIATION

For the initiation of carcinogenesis, a permanent genetic alteration that will be passed on to the progeny of the initiated cell must occur. DNA modification must be sufficiently tenacious to escape efficient repair processes, but not so excessive that cell death results. Many of the gross genetic lesions induced by oxidants will be toxic; however, deletion or rearrangement of promoter and enhancer regions can mediate gene deregulation in surviving cells, a likely consequence of which is gene inactivation. Allelic deletion can also occur through oxidative mechanisms. The inactivation/loss of certain tumour suppressor genes can lead to the initiation and/or progression of carcinogenesis. Tumour suppressor genes can also be inactivated by the alteration of a single critical base pair in the DNA sequence. A common site for point mutations in both the *p53* and retinoblastoma tumour suppressor genes are G-C base pairs in CpG dinucleotide sequences.[31, 32] Similarly, cellular genes can be converted into oncogenes through a single base pair change, and G-C base pairs provide a common target for activating point mutations. For example, the most frequent site of mutation in the *ras* family of oncogenes are G-C base pairs in codons 12 and 13.[33] G-C base pairs

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

in both tumour suppressor genes and oncogenes may therefore represent a vulnerable target for mutation by oxidative stress. The initiating capacity of oxidants may be primarily due to their induction of base changes in the DNA sequence of these genes.

G-C SITES: TARGETS OF OXIDATIVE DNA DAMAGE

The propensity of oxidative stress to induce mutations at G-C sites that persist following replication and repair has been demonstrated in a mutation-reporter plasmid system by Moraes and coworkers.[34, 35] When monkey CV-1 cells were exposed to hydrogen peroxide following plasmid transfection, sequence analysis of the plasmid *supF* locus revealed that 97% of the base changes occurred at G-C sites, with the predominant base change a G-C →A-T transition mutation. Further, a mutational hotspot of polycytosines was identified. Interestingly, hydrogen peroxide increased the quantity, but did not change the spectrum of endogenously produced mutations, suggesting that spontaneous mutagenesis may be continuously occurring through oxidative mechanisms. Other agents whose DNA-damaging actions are thought to be mediated by active oxygen species also produce the same pattern of DNA damage as arises spontaneously. Such is the case with γ-radiation-induced and spontaneous mutations at the hamster *aprt* locus. Single base substitutions were the most common lesion in both cases, with G-C base pairs the predominant substitution target.[36, 37]

The prevalence of mutations at G-C base pairs in the DNA has not been uniformly found in all model systems. For example, when Moraes and coworkers exposed the mutation reporter plasmid to hydrogen peroxide in the presence of iron prior to transfection into CV-1 cells, the predominant lesions were found at A and T sites.[34] Spontaneous mutation of the plasmid, however, occurred primarily at G-C sites. The addition of exogenous transition metals in this in vitro exposure of DNA to oxidants may account for the shift in base specificity for mutations. Other investigators have similarly noted that the spectrum of modified bases is dependent upon the radical-generating system employed, particularly the transition metal utilized to promote generation of hydroxyl radicals. The advent of sensitive gas chromatography/mass spectrometry techniques has enabled quantitative analyses of all oxidized bases, without requiring prior labelling of the DNA. While the spectrum of oxidized products is variable depending on oxidant treatment, detailed analyses by the laboratories of Halliwell and Dizdaroglu[27, 28, 38] have indicated that under biologically relevant exposure conditions, a predominance of G and C modifications are produced. The oxidized products of A and T bases are not without consequence, however; a

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

Salmonella tester strain with A-T base pairs at the site of mutation was sensitive to a variety of oxidative mutagens.[39] Thus while mutagenic lesions at G-C appear to predominate in model systems approximating in vivo exposure to oxidative stress, manipulation of the treatment conditions can activate other mechanisms of mutation.

COPPER IONS BOUND TO DNA MAY FACILITATE OXIDATIVE DNA DAMAGE

The addition of exogenous metals to DNA in solution can promote the production of active oxygen species that can damage bases in DNA. As compared with iron, the addition of copper to DNA in vitro induces more mutations[40] and also mediates more extensive DNA base damage in the presence of oxidants[28]. Native DNA contains tightly bound copper ions.[41] DNA shows a high specificity for the binding of copper as compared to other metal ions.[42] The binding of copper to DNA is proposed to serve several physiological functions (reviewed in Reference 43). Copper may stabilize the scaffolding structure of DNA when histones have dissociated, during both transcriptional activation of genes and DNA synthesis in metaphase. In fact, copper can mediate the renaturation of DNA from single strands in vitro.[44] Through coordinate binding, copper may also provide a linker between DNA and associated nuclear proteins; these copper links may be established during biosynthesis of the DNA molecule. An additional function of bound copper may be to provide a redox signal for DNA stability: a change of valence from Cu(I) to Cu(II) can mediate localized conformational B-to-Z conversion.

Copper can interact directly with the bases of DNA, and a large body of evidence suggests that copper is specifically bound to DNA at G-C sites.[44] For example, Prutz and coworkers demonstrated that double-stranded DNA of alternating G/C base pairs formed complexes with copper of comparable stability and absorption spectra to native DNA complexed with copper.[43] By contrast, DNA with an alternating A/T sequence did not form a stable complex with copper. Further, when DNA of the two sequences was mixed, copper was transferred from DNA of alternating A/T bases to that of alternating G/C bases.

While the binding of copper is necessary for DNA stabilization and function, bound copper ions may provide an adventitious site for deleterious redox reactions. In the presence of copper, a metal-catalyzed redox cycling of electrons can mediate the production of the highly unstable hydroxyl radical from the less reactive superoxide and hydrogen peroxide. Copper may elaborate other free radical species in this sensitive biological target as well. Benzoyl peroxide, a widely used over-the-

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

counter acne medication, undergoes copper-mediated activation to the benzoyloxyl radical, a DNA-damaging species;[45] in a mutation-reporter plasmid, mutagenesis by benzoyl peroxide was copper-dependent and occurred specifically at G-C sites.[46] Copper-mediated production of free radical species at G-C sequences may therefore account for the selective oxidative modification of G and C DNA bases.

8-oxo-G: MEDIATOR OF MUTAGENESIS BY OXIDATIVE STRESS

Quantitatively, the 8-hydroxylated derivative of dG is one of most prevalent oxidized products of DNA generated in vitro under physiological conditions.[27, 28] This lesion also functions as a sensitive biomarker of oxidative DNA damage in vivo, despite that technical considerations to prevent its artifactual production must be addressed.[47] Numerous investigations have indicated that 8-hydroxy-G is formed under various experimental carcinogen treatment protocols (reviewed in Reference 48). Dose-dependent production of 8-hydroxy-G also occurs in human cells in culture, whole animals, or bacteria exposed to ionizing radiation.[49] Further, the excretion of 8-hydroxy-G is correlated with age, metabolic rate, caloric intake, and antioxidant content of the diet.[50–52]

Chemical modification of guanine, such as by oxidation, can affect the configuration of this base in DNA and alter its base-pairing properties. Such alterations can compromise the fidelity of a template containing this base during DNA replication. Introduction of an oxygen atom at the C8 position of G changes the electronic properties of this DNA base. In particular,[15] N-NMR studies of the tautomerism of 8-hydroxy-G indicate that the oxygen at the C8 position is most likely to adopt the keto form at physiological pH. Further, in contrast to its normal counterpart, the oxidized base assumes the *syn* conformation about the glycosyl bond in DNA.[53] Both of these structural considerations impact the base-pairing properties of the oxidized base; NMR structural analyses have demonstrated the 8-oxo-G(*syn*) will form a stable base pair with A(*anti*) in the interior of the DNA helix without perturbation of flanking base pairs.[54] Figure 2 shows the structure and base pairing of G, the formation of its 8-oxidized product (termed 8-oxo-G), and the abnormal base-pair 8-oxo-G can form in DNA.

8-oxo-G was first demonstrated to be mutagenic in vitro by Kuchino and coworkers.[55] Further studies have indicated that DNA polymerases selectively incorporate C and A opposite 8-oxo-G[56] in keeping with the base-pairing properties of the oxidized base. Extension past 8-oxo-G(*syn*)-A(*anti*) proceeds more efficiently than past 8-oxo-G(*anti*)-

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

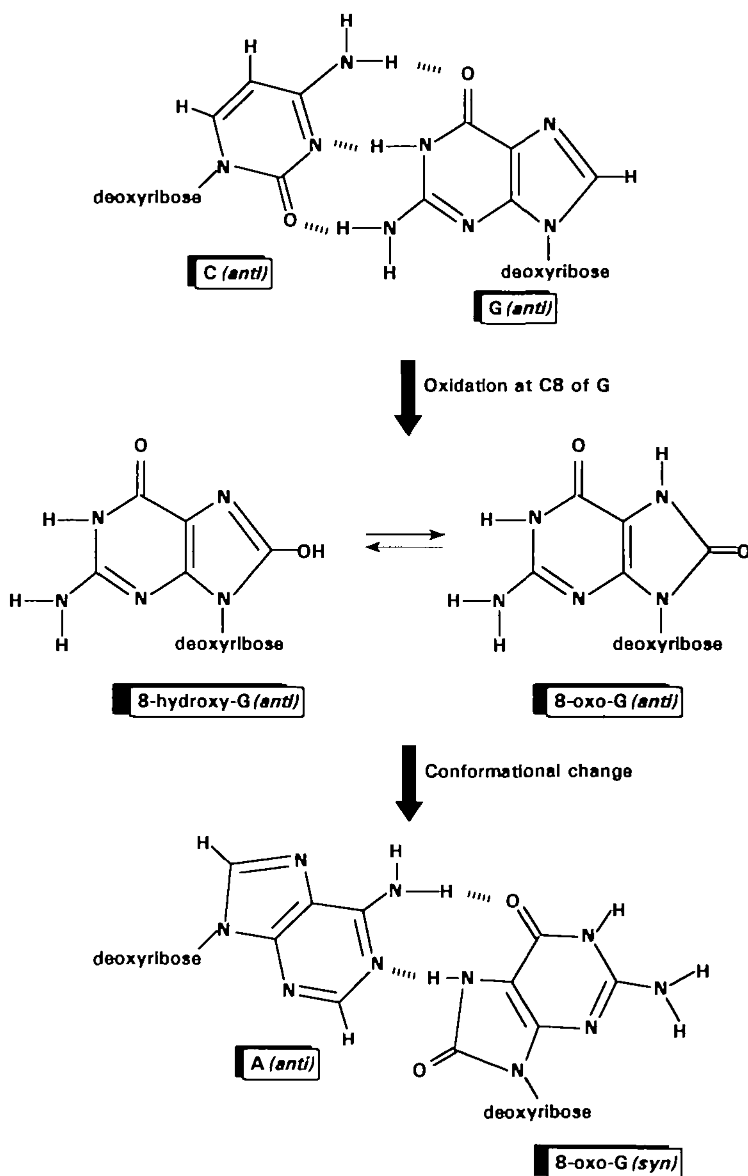

Fig. 2 Oxidation at C8 causes structural and conformational alterations in G that can result in mispairing of 8-oxo-G with A.

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

C(*anti*), suggesting that once misincorporation occurs, the stable G-A mispair is not recognized by proofreading functions and will persist as a mutation. The mutation resulting from this 8-oxo-G-A mispairing will be a G→T transversion. Indeed, site-specific mutagenesis techniques in *Escherichia coli*, wherein 8-oxo-G was incorporated into viral[57] or gapped plasmid[58] DNA, targeted G→T mutations at the sites of the lesions. Further, two mutant strains of *E. coli*, in which spontaneous levels of G→T transversions are increased 100 to 10,000 fold over wild type levels, were found to be defective in cellular mechanisms for eliminating 8-oxo-G. The *mutM* strain lacks Fapy-DNA glycosylase;[59] the primary activity of this enzyme has been demonstrated to be the cleavage of 8-oxo-G, rather than FapyG, prior to replication.[60] The *musT* strain is deficient in the enzyme that specifically hydrolyses 8-oxo-G from the nucleotide pool so that it cannot be incorporated during DNA synthesis.[61]

The existence of 2 separate mechanisms in bacteria that address 8-oxo-G underscores the likely importance of this oxidized base as a mediator of mutagenesis. Analogous cellular mechanisms may operate in mammals; the disappearance of 8-oxo-G over time following whole-body radiation indicates that this lesion is being repaired in mice.[49] Confirmation that 8-oxo-G undergoes stable mispairing with A, coupled with data that this lesion specifically leads to G→T transversion mutations, strengthens the potential role of this oxidative lesion in carcinogenesis by oxidants.

OTHER MECHANISMS OF OXIDATIVE MUTATIONS

While the generation of 8-oxo-G may account for the formation of G→T transversions through mispairing with A, this lesion is not likely to participate in all types of oxidative mutations. Like G, C is a preferred site for binding of copper to DNA, and therefore a primary target for oxidative modification, and oxidative products of this base are formed in vitro and in vivo. In fact, the burden of 5-hydroxy-C produced upon exposure of DNA in vitro to oxidative stress was several fold higher than the burden of 8-oxo-G.[62] Similarly, a greater amount of 5-hydroxy-C than 8-oxo-G was found by analyses of biological samples from a variety of tissues and species. The potential mutagenicity of this lesion has not yet been explored. The development of methodology to specifically generate 5-hydroxy-C and other oxidatively modified products of C will enable examination of the mutagenic capacity of these lesions in test systems. In particular, the oxidation of C may lead to altered base pairing in an analogous manner to the mispairing of 8-oxo-G to A. Moreover, oxidized products of both G and C may function in

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

mutagenesis through other mechanisms besides mispairing, including abnormal interactions with the DNA replication machinery.

The physiological fate of other oxidatively modified bases in the DNA is discussed in a review by Breimer.[23] Thymine glycol is toxic if not excised by a DNA glycosylase prior to replication. Ring-fragmented bases, including FapyG, similarly result in toxicity by blocking replication of DNA. The cleavage of these non-mutagenic oxidative lesions from DNA can, however, lead to the generation of abasic sites, which can in turn cause mutagenesis. Thus the potential for the oxidation of all bases to lead to mutations cannot be ignored.

OVERVIEW OF ROLE OF FREE RADICALS IN TUMOUR PROMOTION

Substantial evidence has established a role for oxidative stress in the later stages of carcinogenesis, the subject of many recent reviews.[63–67] Much of the evidence supporting the hypothesis that free radicals can and do mediate tumour promotion is indirect. In particular, many studies have demonstrated the ability of antioxidants and oxidant detoxifiers to inhibit a wide diversity of promotion-related biochemical processes. These results suggest that free radical processes play substantial roles in the many biochemical and molecular changes leading to tumour promotion. Additional experimental evidence supporting the premise that free radicals can participate in promotion includes the demonstration that tumour promoters can create an environment of oxidative stress. Promoters provoke a rapid and sustained decrease in cellular antioxidant defenses, including superoxide dismutase, catalase, and glutathione peroxidase activities. Further, free radical generating compounds, including a variety of peroxides and hydroperoxides, have demonstrated tumour promoting activity in vivo and can effect cellular changes relevant to tumour promotion. The continuing investigation of the in vitro and in vivo effects of hydroperoxide tumour promoters and active oxygen generating systems has provided much insight into the mechanisms through which free radicals and other oxidants can invoke changes in gene expression leading to tumour promotion.

CYTOTOXICITY AND GROWTH STIMULATION INTERPLAY IN OXIDANT TUMOUR PROMOTION

Tumour promotion in vivo is comprised of a complex series of molecular events. The development of appropriate in vitro models to study mechanisms through which oxidants mediate this stage of carcinogenesis is complicated by the ability of tumour promoters to cause multiple effects simultaneously. In particular, the influence of the promoter on

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

both initiated and normal cells must be considered. In tumour promotion, the initiated cell population is stimulated to expand while the normal population is not. This effect can be achieved either directly, by providing a growth stimulus to the initiated cells, or as the indirect result of the effects of the promoter on the normal population. By selectively stimulating terminal differentiation or toxicity in the normal population, tumour promoters can induce 'compensatory proliferation', wherein the initiated cells proliferate to fill the void left by the removal of their normal counterparts. The phorbol ester tumour promoters have been demonstrated to stimulate initiated cells through both direct and indirect mechanisms simultaneously. These compounds provide a cellular signal that at once stimulates proliferation in the initiated cell, while triggering terminal differentiation in the surrounding normal cells. Oxidative stress may similarly be able to provide this type of dual signal.

The initiation process may confer one or more phenotypic changes in the initiated cell that permits an altered response to tumour promoters, divergent from the reaction of their normal neighbours. For example, the basis for the differential effect of phorbol esters on initiated skin may lie in the fact that initiated skin cells are resistant to calcium-stimulated terminal differentiation while normal cells are not.[68] An analogous mechanism, in which initiated cells are more resistant to the toxicity of oxidants, may participate in oxidant-mediated tumour promotion. Two clones of the murine epidermal skin cell line JB6, which were determined to be promotable or non-promotable by a variety of tumour promoters, have provided a model to study this possibility. While active oxygen stimulated growth of a promotable clone (clone 41), active oxygen was strongly cytostatic to a non-promotable clone (clone 30).[69] The 'promotable' characteristic in vitro may be analogous to 'initiated' in vivo; mouse keratinocytes from initiated skin or papillomas were more resistant to radical-mediated toxicity than were normal keratinocytes.[70] Active oxygen-induced toxicity is mediated through deregulation of intracellular calcium ions (reviewed in Reference 30) resulting from oxidation of cellular membranes and proteins. Certainly the cellular levels of antioxidant defenses influence the extent of toxicity. Indeed, the promoter-sensitive clone 41 was found to have increased levels of glutathione, superoxide dismutase, and catalase.[71] The superior ability of initiated cells to withstand the toxic insult of carcinogens may participate in the neoplastic process in other tissues as well. In fact, the concept that enhanced defenses against reactive intermediates afford a selective advantage to initiated cells originated in models of hepatocarcinogenesis.[72]

While selective toxicity by oxidants may function in tumour promotion, toxicity alone may be insufficient to induce this stage of

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

carcinogenesis.[73] The direct induction of mitogenesis in target initiated cells may complement the cytostatic and cytotoxic effects of oxidants on normal cells in tumour promotion. Active oxygen can stimulate growth in JB6 cells[69] and in fibroblasts.[74] The mitogenic response initiated by a variety of stimuli has been shown to be mediated by the induction of a common pool of genes (reviewed in Reference 75). Among these are the 'primary response genes', whose induction can be stimulated in the absence of protein synthesis. Active oxygen has been demonstrated to transiently enhance the expression of genes of this class, including *c-fos*, *c-jun*, *c-myc* and β-actin.[76, 77] c-*myc* is required for the acquisition of competence and cell proliferation. The c-*fos* gene appears to be located at a point of convergence of multiple signal transduction pathways and is crucial to the cellular response to proliferative stimuli.[78] The protein products of c-*fos* and c-*jun* form a heterodimer through a leucine zipper. This transcription factor activator protein (AP-1) plays a pivotal role in the regulation of the expression of many genes. Transcriptional activation of these primary response genes is a necessary component of oxidant-induced growth stimulation. *jun* and *fos* have provided appropriate model genes to study mechanisms of transcriptional regulation by oxidants that may participate in tumour promotion.

OXIDATION-REDUCTION MECHANISMS FUNCTION IN GENE REGULATION

The potential of oxidative stress to function as a modulator of gene expression has been underscored by recent evidence that a diversity of genes are regulated through redox mechanisms. For example, the interaction of AP-1 with DNA is subject to redox control. The binding of AP-1 to DNA in vitro can be controlled by the oxidation-reduction of a single cysteine residue in the DNA binding domain of the Fos and Jun proteins.[79] Because the AP-1 complex can activate numerous genes, especially those essential to proliferation, interference with its redox control can have wide-ranging effects.

Redox mechanisms have been shown to participate in the regulation of several other mammalian trasncriptional regulators, including protein products of other oncogenes. For example, in vitro DNA binding of the BZLF1[80] and v-Rel oncoproteins are controlled by redox changes, the latter mediated through a conserved cysteine residue.[81] The Rel family of oncoproteins regulate the expression of genes with κB motifs in their promoters. Many of these genes, including IL2Rα, function in lymphoid cell differentiation. v-Rel may interfere with the induction of these genes by other members of the Rel family, through either competitive

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

binding to κB DNA regions or formation of inactive complexes with other Rel proteins. Oxidation-reduction mechanisms may function in both of these inhibitory effects, by mediating protein-protein as well as and protein-DNA interactions. Mammalian regulatory protein-RNA interactions may also be subject to redox control; interaction of iron-responsive element RNA motifs with specific binding proteins is activated upon reduction of a sulfhydryl moiety in the protein.[82]

Redox mechanisms of gene regulation have apparently been conserved throughout evolution. For example, hydrogen peroxide can activate protein binding to the DNA heat-shock regulatory element in both *Drosophila* and in mouse 3T6 fibroblasts.[83] The rapid induction of binding by hydrogen peroxide suggests that direct oxidation may control interaction of this putative transcription factor with promoter DNA. Bacterial regulation of gene expression can also be mediated through redox mechanisms. Many bacterial proteins are synthesized in response to oxidative stress, 9 of which are controlled by a single regulon, *oxy*R.[84] The regulatory signal for the transcriptional activation of genes by *oxy*R has been demonstrated to be the direct and reversible oxidation of the *oxy*R protein.[85]

OXIDATIVE MODIFICATION OF CYSTEINE RESIDUES BY TUMOUR PROMOTERS

Cysteine residues on key regulatory proteins may play a prominent role in the redox control of many genes, including oncogenes. These sulfhydryl moieties are subject to oxidative modification by reactive intermediates formed from tumour promoters. For example, the in vivo tumour promoting activity of butylated hydroxytoluene hydroperoxide has been shown to require the formation of a quinone methide.[86] A soft electrophile, this quinone methide is likely to preferentially interact with sulfhydryls in cysteine rather than nitrogen atoms in amino acids or nucleic acids.[87] Transcriptional regulators represent one possible target for oxidative sulfhydryl modification by this tumour promoter; other alternatives include proteins controlling central signalling pathways. The function of proteins regulating calcium ion homeostasis, for example, can be impaired by sulfhydryl modification. The intracellular sites of interaction of the quinone methide derived from butylated hydroxytoluene hydroperoxide, as well as the mechanisms through which it stimulates tumour promotion-related cellular events, are currently under investigation.

Cysteine modification may also occur as a consequence of intracellular glutathione depletion in conditions of oxidative stress. When the protection offered by glutathione is compromised, sulfhydryl moi-

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

eties on regulatory proteins are more vulnerable to oxidation. In fact, glutathione depletion can signal induction of the oxidatively regulated mammalian gene heme oxygenase.[88] Heme oxygenase shows a common pattern of increased expression in response to a variety of oxidative signals, from oxygen radicals to sulfhydryl modifiers.[89] Further, the responsiveness of this gene to oxidants has been demonstrated in cell types of diverse tissue and species origin, from human skin to rodent lung.[90] The universality of heme oxygenase induction suggests a critical role for this gene in the response to oxidative stress. The mechanisms through which cellular cysteine residue oxidation can mediate induction of this gene, including the role of specific genetic regulatory elements in controlling expression, are being studied by Tyrrell and coworkers. Cysteine residue oxidation may function in the regulation of other genes as well, and may play a significant role in oxidative modulation of gene expression by tumour promoters.

CELLULAR MEDIATORS OF OXIDANT MODULATION OF GENE EXPRESSION

The ability of oxidants to activate signal transduction and induce other cellular effects involved in the regulation of gene expression has been extensively studied by Cerutti and coworkers in JB6 cells. The rise in intracellular calcium stimulated by active oxygen in these cells may be a first and necessary step to other oxidant-induced cellular changes.[91] Control of intercellular calcium ion concentration is essential for cellular function. While excessive rises in intracellular calcium can lead to toxicity, transient deregulation of calcium ion sequestration can mediate many cellular changes, including the induction of gene expression and intercellular communication, both of which may participate in tumour promotion by oxidants.

A rise in intracellular calcium may mediate DNA strand breakage and poly-ADP ribosylation by oxidative stress; both of these effects can be inhibited by Quin 2, a calcium chelator.[69] The production of strand breaks causes local changes in chromatin structure and can stimulate poly-ADP ribosylation of histones[92] thereby augmenting the distortion of chromatin conformation. This distortion may activate c-*fos*[93] and other early response genes in the absence of other signals. DNA damage by oxidants can enhance expression of other mammalian genes, including those of the *gadd* (Growth Arrest and DNA Damage) family.[94] While the *gadd* genes are thought to function in a regulatory capacity, the relationship between induction of these genes and the process of tumour promotion has not been studied. However, other regulatory

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

genes relevant to tumour promotion may similarly be activated by the production of strand breaks.

Modulation of calcium homeostasis by active oxygen can activate protein kinase pathways as well. Phosphorylation of ribosomal protein S6, which has a postulated role in the stimulation of protein synthesis and the acquisition of growth competence, is also initiated by active oxygen.[95] It is hypothesized that an oxidant-stimulated rise in intracellular calcium activates a calcium/calmodulin-dependent protein kinase, which subsequently phosphorylates and activates an S6 kinase. Oxidative stress also activates protein kinase C,[96] a known regulator of many intracellular processes, including those related to growth and differentiation. The mechanism of activation may involve both a rise in intracellular calcium and direct oxidation. Oxidant-stimulated translocation of protein kinase C to the plasma membrane was calcium-dependent, but occurred without stimulation of kinase activity. The in vitro phosphorylating capacity of protein kinase C was instead stimulated by mild oxidant treatment or through oxidative sulfhydryl modification. In other systems, protein kinase C can be activated by mild oxidation of the regulatory domain, and subsequently inactivated by further oxidation.[97] Thus direct sulfhydryl oxidation and deregulation of calcium ion homeostasis can interplay in the activation of protein kinase C. The convergence of other cellular effects of oxidants may similarly contribute to their capacity to modulate signal transduction pathways.

Transcriptional activation of c-*fos* by active oxygen species requires not only activation of kinase pathways, but also AP-1 binding. In a study of the genetic regulatory elements that mediate c-*fos* induction by active oxygen species, the joint dyad symmetry element-AP-1 motif exerted the most potent enhancer effect.[98] Oxidative stress stimulated binding, mediated by Fos-Jun, to this enhancer region and to the AP-1 consensus sequence in mobility shift assays. In addition to kinase activation, poly-ADP-ribosylation was necessary for binding to AP-1 and for transcriptional activation of c-*fos*. Since the Fos protein was not ADP-ribosylated, the function of ADP-ribosylation may be to repair DNA strand breaks so that transcription can proceed.

The expression of other genes playing pivotal roles in the cellular response to oxidative stress occurs subsequent to the activation of signalling pathways and primary response genes, such as c-*fos*. Among the genes controlled through AP-1 is ornithine decarboxylase, which catalyzes the first and rate-limiting step in polyamine biosynthesis. Oxidant tumour promoters can stimulate a rapid and transient induction of this gene. While the cellular mechanisms leading to this induction have not been discerned, the enhanced expression of c-*fos* or c-*jun* may function to regulate this gene. The specific function of polyamines

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

remains unclear; however, their production is essential for proliferation and tumour promotion.

It appears that oxidants can utilize several different avenues to alter cellular function. The cellular effects of oxidants on signal transduction pathways and other important cellular regulatory processes can lead to the modulation of the expression of a seemingly extensive battery of genes. A specific role for all genes induced by oxidative stress in either the toxic or proliferative effects of oxidants (which may be relevant to tumour promotion) has not been found. However, the diversity of genes and gene products which can be affected by oxidants supports the significance of oxidative stess as a cellular regulator. That oxidants can participate in normal cell function indicates that alteration of the oxidant status can have wide-ranging effects, including the alteration of gene expression in tumour promotion.

FREE RADICALS CAN MEDIATE MALIGNANT CONVERSION

By selectively modifying the pattern of gene expression in initiated cells, tumour promoters can elicit the production of clonally-derived benign growths. These tumours can be converted into rapidly-growing malignant neoplasms through further DNA damage. The spontaneous conversion of the multiple skin papillomas generated in the classical initiator-promoter regimen to malignant carcinomas is uncommon. Instead, the production of cancers from benign tumours can be experimentally achieved by treatment of papillomas with either an initiator[99] or a free radical-generating agent that can elicit DNA damage, such as benzoyl peroxide.[100] Treatment with such converting agents may result in further direct DNA base modification or the transposition of genetic material. A second, heritable genetic lesion is thus produced in one or more cells in the benign tumour, leading to irreversible transformation into an autonomously growing cancer.

SUMMARY

Exposure to active oxygen species in aerobic organisms is continuous and unavoidable. In fact, oxidation-reduction mechanisms have been widely adapted to function in and regulate cellular processes. The effects of an oxidative environment are not without pernicious consequences, however. DNA base oxidation may proceed continually. Chromatin may offer some protection against oxidative DNA damage[101] and specific repair processes may prevent or correct oxidative mutations. However, oxidants from both endogenous and exogenous sources can overwhelm these defenses and mutagenesis can and does occur. Similarly, defense against oxidation of other biomolecules, including mem-

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

branes and proteins, may prove insufficient when exposure to active oxygen species is severe or long in duration. The condition of oxidative stress that ensues can cause multiple cellular effects and can elicit all stages of carcinogenesis.

ACKNOWLEDGEMENTS

The support of the National Institutes of Health (ESO7141, ESO3819 and CA 44530) is gratefully acknowledged.

REFERENCES

1 Ames BN. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science 1983; 221: 1256–1264.
2 Willett WC, MacMahon B. Diet and cancer—an overview. N Engl J Med 1984; 310: 633–638.
3 Kensler TW, Bush DM, Kozumbo WJ. Inhibition of tumor promotion by a biomimetic superoxide dismutase. Science 1983; 221: 75–77.
4 Salim AS. Removing oxygen-derived free radicals delays hepatic metastases and prolongs survival in colonic cancer: a study in the rat. Oncology 1992; 49: 58–62.
5 Perchellet J-P, Perchellet EM. Antioxidants and multistage carcinogenesis in mouse skin. Free Radical Biol Med 1989; 7: 377–408.
6 Nelson RL. Dietary iron and colorectal cancer risk. Free Radical Biol Med 1992; 12: 161–168.
7 Stevens RG, Nerishi K. Iron and oxidative damage in human cancer. In: Spatz L, Bloom AD, eds. Biological consequences of oxidative stress: implications for cardiovascular disease and carcinogenesis. New York: Oxford University Press, 1992; pp138–161.
8 Weitzman SA, Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. Blood 1990; 76: 655.
9 Trush MA, Kensler TW. Role of free radicals in carcinogen activation. In: Sies H, ed. Oxidative stress: oxidants and antioxidants. London: Academic Press, 1991; pp277–318.
10 Rous P, Kidd JG. Conditional neoplasms and subthreshold neoplastic states: a study of the tar tumours of rabbits. J Exp Med 1941; 73: 365–389.
11 Mottram JC. A developing factor in experimental blastogenesis. J Pathol Bacteriol 1944; 56: 181–187.
12 Berenblum I, Shubik P. The role of croton oil application associated with a single painting of a carcinogen, in tumour induction of the mouse's skin. Br J Cancer 1947; 1: 379–382.
13 Cerutti PA. Response modification creates promotability in multistage carcinogenesis. Carcinogenesis 1988; 9: 519–526.
14 Harris C. Individual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. Carcinogenesis 1989; 10: 1563–1566.
15 Fenn WO, Gerschman R, Gilbert DL, Terwilliger DE, Cothran FV. Mutagenic effects of high oxygen tension on Escherichia Coli. Proc Natl Acad Sci USA 1957; 43: 1027–1032.
16 Parshad R, Sanford KK. Oxygen supply and stability of chromosomes in mouse embryo cells in vitro. J Natl Cancer Inst 1971; 47: 1033–1035.
17 Hoffman ME, Meneghini R. Action of hydrogen peroxide on human fibroblasts in culture. Photochem Photobiol 1979; 30: 151–155.
18 Weitberg AB, Weitzman SA, Destrempes M, Latt SA, Stossel TP. Stimulated human phagocytes produce cytogenetic changes in cultured mammalian cells. N Engl J Med 1983; 308: 26–30.

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

19 Ziegler-Skylakakis K, Andrae U. Mutagenicity of hydrogen peroxide in V79 Chinese hamster cells. Mutat Res 1987; 192: 65–67.
20 Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide in vitro. Proc Natl Acad Sci USA 1992; 89: 3030–3034.
21 Weitzman SA, Weitberg AB, Clark EP, Stossel TP. Phagocytes as carcinogens: malignant transformation produced by human neutrophils. Science 1985; 227: 1231–1233.
22 Inoue S Kawanishi S. Hydroxyl radical production and human DNA damage induced by ferric nitrilotriacetate and hydrogen peroxide. Cancer Res 1987; 47: 6522–6527.
23 Breimer LH. Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: the role of DNA base damage. Mol Carcinog 1990; 3: 188–197.
24 Lesko SA, Lorentzen RJ, Ts'O POP. Role of superoxide in deoxyribonucleic acid strand scission. Biochemistry 1980; 19: 3023–3028.
25 Brawn K, Fridovich I. DNA strand scission by enzymically generated oxygen radicals. Arch Biochem Biophys 1981; 206: 414–419.
26 Nassi-Calò L, Mello-Filho AC, Meneghini R. o-phenanthroline protects mammalian cells from hydrogen peroxide-induced gene mutation and morphological transformation. Carcinogenesis 1989; 10: 1055–1057.
27 Aruoma OI, Halliwell B, Dizdaroglu M. Iron-ion-dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthine oxidase. J Biol Chem 1989; 264: 13024–13028.
28 Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M. Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochem J 1991; 273: 601–604.
29 Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: its mechanism and measurement in mammalian systems. FEBS Lett 1991; 281: 9–19.
30 Nicotera P, Bellomo G, Orrenius S. The role of Ca+2 in cell killing. Chem Res Toxicol 1990; 3: 484–494.
31 Nigro J, Baker S, Preisinger A, et al. Mutations in the p53 gene occur in diverse human tumor types. Nature 1989; 342: 705–708.
32 Yandell D, Campbell T, Dayton S, et al. Oncogenic point mutations in the human retinoblastoma gene: their application to genetic counseling. N Engl J Med 1989; 321: 1689–1695.
33 Bos J. The ras gene family and human carcinogenesis. Mutat Res 1988; 195: 255–271.
34 Moraes EC, Keyse SM, Tyrrell RM. The spectrum of mutations generated by passage of a hydrogen peroxide damaged shuttle vector plasmid through a mammalian host. Nucleic Acids Res 1989; 17: 8301–8312.
35 Moraes EC, Keyse SM, Tyrrell RM. Mutagenesis by hydrogen peroxide treatment of mammalian cells: a molecular analysis. Carcinogenesis 1990; 11: 283–293.
36 Miles C, Meuth M. DNA sequence determination of gamma-radiation-induced mutations of the hamster *aprt* locus. Mutat Res 1989; 227: 97–102.
37 Phear G, Armstrong W, Meuth M. Molecular basis of spontaneous mutation at the *aprt* locus of hamster cells. J Mol Biol 1989; 209: 577–582.
38 Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M. Damage to the bases in DNA induced by hydrogen peroxide and ferric ion chelates. J Biol Chem 1989; 264: 20509–20512.
39 Levin DE, Hollstein M, Christman MF, Schwiers EA, Ames BN. A new *Salmonella* tester strain (TA102) with A-T base pairs at the site of mutation detects oxidative mutagens. Proc Natl Acad Sci USA 1982; 79: 7445–7449.
40 Tkeshelashvili LK, McBride T, Spence K, Loeb LA. Mutation spectrum of copper-induced DNA damage. J Biol Chem 1991; 266: 6401–6406.
41 Eichorn LE, Shin YA. Interaction of metal ions with polynucleotides and related compounds XII. The relative effect of various metal ions on DNA helicity. J Am Chem Soc 1968; 90: 7323–7328.
42 Sagripanti J-L, Goering PL, Lamanna A. Interaction of copper with DNA and antagonism by other metals. Toxicol Appl Pharmacol 1991; 110: 477–485.

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

43 Prutz WA, Butler J, Land EJ. Interaction of copper(I) with nucleic acids. Int J Rad Biol 1990; 58: 215–234.
44 Pezzano H, Podo F. Structure of binary complexes of mono- and polynucleotides with metal ions of the first transition group. Chem Rev 1980; 80: 365–399.
45 Swauger JE, Dolan PM, Zweier JL, Kuppusamy P, Kensler TW. Role of the benzoyloxyl radical in DNA damage mediated by benzoyl peroxide. Chem Res Toxicol 1991; 4: 223–228.
46 Akman SA, Doroshow JH, Kensler TW. Copper-dependent site-specific mutagenesis by benzoyl peroxide in the *supF* gene of the mutation reporter plasmid pS189. Carcinogenesis 1992; 13: pp1783–1787.
47 Claycamp HG. Phenol sensitization of DNA to subsequent oxidative damage in 8-hydroxyguanine assays. Carcinogenesis 1992; 13: 1289–1292.
48 Floyd RA. The role of 8-hydroxyguanine in carcinogenesis. Carcinogenesis 1990; 11: 1447–1450.
49 Kasai H, Crain PF, Kuchino Y, Nishimura S, Oosuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 1986; 7: 1849–1851.
50 Fraga CG, Shigenaga MK, Park J-W, Degan P, Ames BN. Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. Proc Natl Acad Sci USA 1990; 87: 4533–4537.
51 Simic MG, Bergtold DS. Dietary modulation of DNA damage in human. Mutat Res 1991: 250: 17–24.
52 Simic MG. DNA damage, environmental toxicants, and rate of aging. Environ Carcinog Ecotox Revs 1991; C9: 113–153.
53 Cho BP, Kadlubar FF, Culp SJ, Evans FE. ^{15}N nuclear magnetic resonance studies on the tautomerism of 8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanosine, and other C8-substituted guanine nucleosides. Chem Res Toxicol 1990; 3: 445–452.
54 Kouchakdjian M, Bodepudi V, Shibutani S, et al. NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7*H*-dG) opposite deoxyadenosine in a DNA duplex. 8-oxo-7*H*-dG(*syn*)-dA(*anti*) alignment at lesion site. Biochem 1991; 30: 1403–1412.
55 Kuchino Y, Mori F, Kasai H, et al. Misreading of DNA templates containing 8-hydroxyguanosine at the modified base and at adjacent residues. Nature 1987; 327: 77–79.
56 Shibutini S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxo-dG. Nature 1991; 349: 431–434.
57 Wood ML, Dizdaroglu M, Gajewski E, Essigmann JM. Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochem 1990; 29: 7024–7032.
58 Moriya M, Ou C, Bodepudi V, Johnson F, Takeshita M, Grollman AP. Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in *E. coli*. Mutat Res 1991; 254: 281–2988.
59 Michaels ML, Pham L, Cruz C, Miller JH. MutM, a protein that prevents G-C to T-A transversions, is formamidopyrimidine-DNA glycosylase. Nucleic Acids Res 1991; 19: 3629–3632.
60 Tchou J, Kasai H, Shibutani S, et al. 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. Proc Natl Acad Sci USA 1991; 88: 4690–4694.
61 Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature 1992; 355: 273–275.
62 Wagner JR, Hu C-C, Ames BN. Endogenous oxidative damage of deoxycytidine in DNA. Proc Natl Acad Sci USA 1992; 89: 3380–3384.
63 Cerutti P. Prooxidant states in tumor promotion. Science 1985; 227: 375–381.
64 Kensler TW, Taffe BG. Free radicals in tumor promotion. Adv Free Radical Biol Med 1986; 2: 347–387.

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

65 Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radical Biol Med 1990; 8: 583–599.
66 Frenkel K. Carcinogen-mediated oxidant formation and oxidative DNA damage. Pharmacol Ther 1992; 53: 127–166.
67 Witz G. Active oxygen species as factors in multistage carcinogenesis. Proc Soc Exp Biol Med 1991; 198: 675–682.
68 Kulesz-Martin MF, Koehler B, Hennings H, Yuspa SH. Quantitative analysis for carcinogen altered differentiation in mouse epidermal cells. Carcinogenesis (Lond) 1980; 1: 995–1006.
69 Muehlematter D, Larsson R, Cerutti P. Active oxygen induced DNA strand breakage and poly ADP-ribosylation in promotable and non-promotable JB6 mouse epidermal cells. Carcinogenesis 1988; 9: 239–245.
70 Hartley J, Gibson N, Kilkenny A, Yuspa SH. Mouse keratinocytes derived from initiated skin or papillomas are resistant to DNA strand breakage by benzoyl peroxide: a possible mechanism for tumor promotion by benzoyl peroxide. Carcinogenesis 1987; 8: 1827–1830.
71 Crawford D, Amstad P, Foo DDY, Cerutti P. Constitutive and phorbol-myristate-acetate regulated antioxidant defense of mouse epidermal JB6 cells. Mol Carcinog 1989; 2: 136–143.
72 Solt D, Farber E. New principle for the analysis of chemical carcinogenesis. Nature 1976; 263: 701–703.
73 Weinstein IB. Toxicity, cell proliferation, and carcinogenesis. Mol Carcinog 1992; 5: 2–3.
74 Murrell G, Francis N, Bromley L. Modulation of fibroblast proliferation by oxygen free radicals. Biochem J 1990; 265: 659–665.
75 Herschman HR. Primary response genes induced by growth factors and tumor promoters. Annu Rev Biochem 1991; 60: 281–319.
76 Shibanuma M, Kuroki T, Nose K. Induction of DNA replication and expression of proto-oncogene c-myc and c-fos in quiescent Balb/3T3 cells by xanthine/xanthine oxidase. Oncogene 1988; 3: 17–21.
77 Crawford D, Amstad P, Zbinden I, Cerutti PA. Oxidant stress induces the protooncogenes c-fos and c-myc in mouse epidermal cells. Oncogene 1988; 3: 27–32.
78 Curran T. The fos oncogene. In: Reddy EP, Skalka AM, Curran T, eds. The oncogene handbook. Amsterdam: Elsevier, 1988; pp307–325.
79 Abate C, Patel L, Rauscher FJ, III, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. Science 1990; 249: 1157–1161.
80 Bannister AJ, Cook A, Kouriades T. *In vitro* DNA binding activity of Fos/Jun and BZLF1 but not C/EBP is affected by redox changes. Oncogene 1991: 6: 1243–1250.
81 Kumar S, Rabson AB, Galinas C. The RxxRxRxxC motif conserved in all Rel/kB proteins is essential for the DNA-binding activity and redox regulation of the v-Rel oncoprotein. Mol Cell Biol 1992; 12: 3094–3106.
82 Hentze MW, Rouault TA, Harford JB, Klausner RD. Oxidation-reduction and the molecular mechanism of a regulatory RNA-protein interaction. Science 1989; 244: 357–359.
83 Becker J, Mezger V, Courgeon A-M, Best-Belpomme M. Hydrogen peroxide activates immediate binding of a *Drosophila* factor to DNA heat-shock regulatory element *in vivo* and *in vitro*. Eur J Biochem 1990; 189: 553–558.
84 Christman MF, Morgan RW, Jacobson FS, Ames BN. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell 1985; 41: 753–762.
85 Storz G, Tartaglia LA, Ames BN. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science 1990; 248: 189–194.
86 Guyton KZ, Bhan P, Kuppusamy P, Zweier JL, Trush MA, Kensler TW. Free radical-derived quinone methide mediates skin tumor promotion by butylated hydroxytoluene hydroperoxide: expanded role for electrophiles in multistage carcinogenesis. Proc Natl Acad Sci USA 1991; 88: 946–950.

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

87 Bolton JL, Valerio LG, Thompson JA. The enzymatic formation and chemical reactivity of quinone methides correlate with alkylphenol-induced toxicity in rat hepatocytes. Chem Res Toxicol 1992; 5: 816–822.
88 Lautier D, Luscher P, Tyrrell RM. Endogenous glutathione levels modulate both constitutive and UVA radiation/hydrogen peroxide inducible expression of the human heme oxygenase gene. Carcinogenesis 1992; 13: 227–232.
89 Keyse SM, Tyrrell RM. Induction of the heme oxygenase gene in human skin fibroblasts by hydrogen peroxide and UVA (365) radiation: evidence for the involvement of the hydroxyl radical. Carcinogenesis 1989; 11: 787–791.
90 Applegate LA, Luscher P, Tyrrell RM. Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. Cancer Res 1991; 51: 974–978.
91 Cerutti P, Larsson R, Kruptiza G, Muehlematter D, Crawford D, Amstad P. Pathophysiological mechanisms of active oxygen. Mutat Res 1989; 214: 81–88.
92 Kruptiza G, Cerutti P. Poly ADP-ribosylation of histones in intact human keratinocytes. Biochemistry 1989; 28: 4054–4060.
93 Stewart AF, Herrera R, Nordheim A. Rapid induction of c-fos transcription reveals quantitative linkage of RNA polymerase II and topoisomerase I enzyme activities. Cell 1990; 60: 141–149.
94 Luethy JD, Farnoli J, Park JS, Fornace AJ, Holbrook NJ. Isolation and characterization of the hamster *gadd153* gene. J Biol Chem 1990; 265: 16521–16526.
95 Larsson R, Cerutti P. Oxidants induce phosphorylation of ribosomal protein S6. J Biol Chem 1988; 263: 17452–17458.
96 Larsson R, Cerutti P. Translocation and enhancement of phosphotransferase activity of protein kinase C following exposure in mouse epidermal cells to oxidants. Cancer Res 1989; 49: 5627–5632.
97 Gopalakrishna R, Anderson WB. Ca^{+2} and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain. Proc Natl Acad Sci USA 1989; 86: 6758–6762.
98 Amstrad PA, Krupitza G, Cerutti PA. Mechanisms of c-fos induction by active oxygen. Cancer Res 1992; 52: 3952–3960.
99 Hennings H, Shores R, Wenk ML, Spangler EF, Tarone R, Yuspa SH. Malignant conversion of mouse skin tumours is increased by tumour initiators and unaffected by tumour promoters. Nature 1983; 304: 67.
100 O'Connell JF, Klein-Szanto AJP, DiGiovanni DM, Fries JW, Slaga TJ. Enhanced malignant progression of mouse skin tumors by the free-radical generator benzoyl peroxide. Cancer Res 1986; 46: 2863–2865.
101 Ljungman M, Hanawalt PC. Efficient protection against oxidative DNA damage in chromatin. Mol Carcinog 1992; 5: 264–269.

Downloaded from http://bmb.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016