REVIEW ARTICLE

# Repression of gene expression by oxidative stress

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Gene expression is modulated by both physiological signals (hormones, cytokines, etc.) and environmental stimuli (physical parameters, xenobiotics, etc.). Oxidative stress appears to be a key pleiotropic modulator which may be involved in either pathway. Indeed, reactive oxygen species (ROS) have been described as second messengers for several growth factors and cytokines, but have also been shown to rise following cellular insults such as xenobiotic metabolism or enzymic deficiency. Extensive studies on the induction of stress-response genes by oxidative stress have been reported. In contrast, owing to the historical focus on gene induction, less attention has been paid to gene repression by ROS. However, a growing number of studies have shown that moderate (i.e. non-cytotoxic) oxidative stress specifically down-regulates the expression of various genes. In this review, we describe the alteration of several physiological functions resulting from oxidative-stress-mediated inhibition of gene transcription. We will then focus on the repressive oxidative modulation of various transcription factors elicited by ROS.

Key words: cysteine, gene regulation, pathophysiology, reactive oxygen species (ROS), transcription factor.

## INTRODUCTION TO OXIDATIVE STRESS

### Cellular redox status

Redox chemistry involves electron exchanges between molecules that display several possible oxidation states according to their own oxidation potential (referred to as $E^0$). This notion is conceptually close to that of p$K_a$, which is associated with acid–base balance. Within the cellular context, the redox status depends on the relative amounts of the oxidized and reduced partners of major redox molecules, such as glutathione. This ratio, GSSG/GSH, reflects the redox status within the cell. It usually averages 1 % ([1] and references therein), which means that GSH prevails over GSSG. Thus the oxidation of a limited amount of GSH into GSSG can dramatically change this ratio (i.e. greatly affect the redox status within the cell). This status may also be assayed using fluorochrome probes (such as 2′,7′-dichlorofluorescein), whose fluorescence changes according to their oxidation state [2]. The glutathione system acts as a homoeostatic redox buffer. This is an important cellular parameter, since the intracellular redox status monitors the relative amounts of the oxidized and reduced species of each redox system within the cell, depending on its oxidation potential ($E^0$). Under oxidative conditions, the oxidized partner predominates. In particular, some transcription factors may be either oxidized or reduced according to the redox status of the cell.

### Reactive oxygen species (ROS)

Owing to its electronic configuration, oxygen is prone to gain electrons and is thus a potent oxidant. However, kinetic considerations limit the reactivity of the dioxygen molecule $O_2$. During the respiration process, $O_2$ is progressively reduced by a controlled supply of four electrons to yield water. However, the incomplete reduction of $O_2$ is possible, and leads to the formation of chemical entities that are still potent oxidants. These molecules are known as ROS. Following a one-, two- or three-electron reduction, $O_2$ may generate successively $O_2^{-\bullet}$ (superoxide radical anion), $H_2O_2$ (hydrogen peroxide) or $OH^\bullet$ (hydroxyl radical). ROS are able to oxidize biological macromolecules such as DNA, protein and lipids [3]. The production of the various radicals is linked via chemical or enzymic reactions (Figure 1). Superoxide dismutase (SOD) converts $O_2^{-\bullet}$ into $H_2O_2$, and the latter can generate $OH^\bullet$ in the presence of $Fe^{2+}$ cations (Fenton reaction). Some chemical reactions and radiations (such as UVA) may drive the dioxygen molecule into an excited state [$^1O_2$], called singlet oxygen (because of its null spin value). It should be noted that nitric oxide (NO) can also be oxidized into reactive nitric oxide species, which may show behaviour similar to that of ROS. In particular the combination of NO and $O_2^{-\bullet}$ can yield a strong biological oxidant, peroxynitrite [4].

Basal cellular metabolism continuously produces ROS. Indeed, the usually well controlled enzymic systems that use electron transfer may undergo leakage. In the presence of $O_2$, any such electron leakage may result in ROS production. This mainly occurs within the mitochondria, where the respiratory chain combines oxygen and electrons [5]. However, it can also happen in other cellular compartments, owing to the activities of oxidases (e.g. NADPH oxidase, xanthine oxidase and monoamine oxidase). For example, following physiological signals that transit through membrane receptors, ROS can be produced within the cell via the activation of an NADPH oxidase (see below). The cytochrome P450 (CYP) mono-oxygenases are also widespread, and may produce ROS using both $O_2$ and electron transport (see below).

Abbreviations used: AhR, aryl hydrocarbon receptor; AP-1, activator protein 1; Arnt, AhR nuclear translocator; CP-1, CCAAT-binding protein 1; CYP, cytochrome P450; DBD, DNA-binding domain; EPO, erythropoietin; ER, oestrogen receptor; GR, glucocorticoid receptor; HIF, hypoxia-inducible factor; HLH, helix–loop–helix; IL-2 (etc.), interleukin 2 (etc.); IRE, iron-responsive element; IRP, iron-regulatory protein; NFI, nuclear factor I; NF-κB, nuclear factor κB; $pO_2$, oxygen partial pressure; PEPCK, phosphoenolpyruvate carboxykinase; Ref-1, redox factor 1; ROS, reactive oxygen species; SOD, superoxide dismutase; TAD, transactivating domain; TNFα, tumour necrosis factor α; Trx, thioredoxin; USF, upstream stimulatory factor; XRE, xenobiotic-responsive element.

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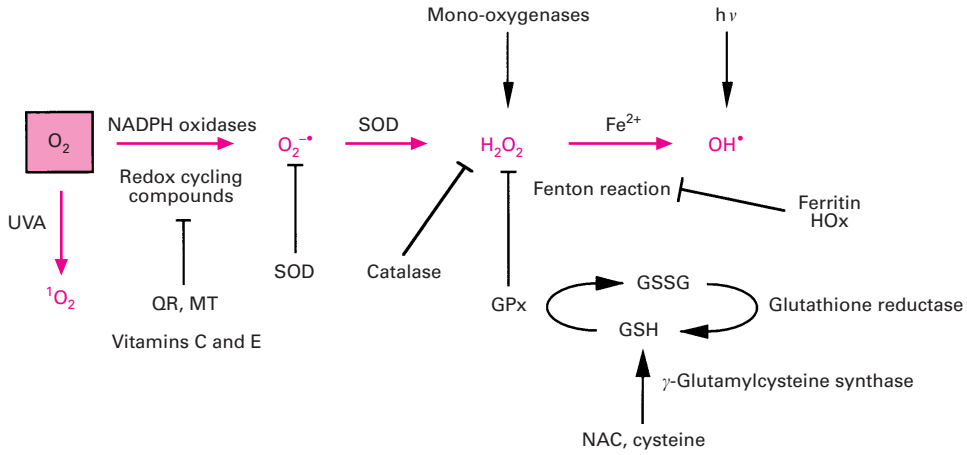


**Figure 1 ROS generation and detoxification**

Various chemical reactions, with or without enzymic catalysis, generate ROS. The dioxygen molecule undergoes successive reductions which yield the superoxide radical anion ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2$) and the hydroxyl radical ($OH^\bullet$). Antioxidant systems act as ROS scavengers to maintain the intracellular redox status. Quinone reductase (QR) detoxifies quinone compounds, metallothionein (MT) traps (heavy) metal cations, and vitamins C and E trap free radicals. SOD and catalase respectively dismutate superoxide (into oxygen and hydrogen peroxide) and hydrogen peroxide (into oxygen and water). Glutathione peroxidase (GPx) acts like catalase on various peroxide compounds, including $H_2O_2$. The catalytic cycle of glutathione peroxidase involves the oxidation of GSH. GSSG can be reduced back to GSH by glutathione reductase. γ-Glutamylcysteine synthase is the limiting enzyme in the synthesis of GSH, and *N*-acetylcysteine (NAC) is a precursor of GSH. Haem oxygenase (HOx) catabolizes free haem structures, and the ferritin molecule traps Fe cations, which limits the deleterious Fenton reaction. hν, symbol for radiation energy.

## Antioxidant systems

As mentioned above, ROS are produced naturally and continuously within the cell. In order to prevent their accumulation and possible deleterious effects, antioxidant systems act as ROS scavengers (Figure 1). Thiol-containing moieties (such as the cysteine residue found in glutathione) have a reducing power (i.e. a low $E^0$). They therefore display antioxidant properties because they can trap ROS (i.e. supply them with electrons, therefore abolishing their oxidative power). The intracellular glutathione content varies within the range 5–10 mM, depending on cell type and cellular compartment. Owing to its ubiquitous prevalence, glutathione acts as an antioxidant buffer within the cell. Moreover, several enzymic systems detoxify ROS: catalase dismutates $H_2O_2$, and SOD eliminates $O_2^{-\bullet}$ (but generates $H_2O_2$). Glutathione peroxidase catalyses the reduction of peroxides (ROOH; including $H_2O_2$) into alcohols (ROH), using the reducing potential of glutathione. The cysteine-rich metallothionein protein also displays antioxidant properties [6]. Other enzymes, including quinone reductase and haem oxygenase, can prevent the formation of oxygen-derived radicals. These enzymes are induced as part of a concerted response to oxidative stress. Cells also protect themselves with antioxidant systems involving a cascade of functional redox molecules, such as thioredoxin (Trx) and redox factor 1 (Ref-1) (see below), or the radical-scavenging vitamins C (cytosol) and E (membrane-bound) (for a review, see [7]). The expression of antioxidant proteins and of enzymes that regenerate them (such as glutathione reductase and Trx reductase) is induced at the transcriptional level (see below) by oxidative stress [8].

## Oxidative stress

'Oxidative stress' occurs when redox homoeostasis within the cell is altered. This imbalance may be due to either an overproduction of ROS or a deficiency in an antioxidant system. Various exogenous stresses may cause an oxidative wave. Energetic radiation can generate hydroxyl radicals (water radiolysis) and singlet oxygen. An increase in oxygen supply (hyperoxia) increases the natural formation of ROS. The stimulation of the immune system may lead to a massive local production of ROS and HClO (hypochlorous acid, the strongest physiological oxidant) because of the activities of an NADPH oxidase and a myeloperoxidase in the phagocytes [9,10]. Moreover, the activity of acyl-CoA oxidase during peroxisomal proliferation may produce substantial quantities of $H_2O_2$ [11]. Some xenobiotics, known as uncoupled substrates, boost the production of ROS by CYPs [12–15]. Quinone compounds (e.g. menadione, adriamycin, mitomycin C) are redox-cycling agents [16]. Molecules containing a metallic cation (Fe, Cu, etc.) may also promote $O_2^{-\bullet}$ formation because they have the ability to store and easily give an electron to molecular dioxygen [17].

The deficiency of an antioxidant system will elicit spontaneous ROS accumulation within the cell. For example, compounds that inhibit key enzymes involved in the synthesis of glutathione or of ROS-scavenging enzymes cause a sustained oxidative stress [18], e.g. buthionine-*S*,*R*-sulphoximine, which inhibits γ-glutamylcysteine synthase. A decrease in the pool of antioxidant vitamins also leads to higher intracellular ROS levels [5].

If the production of ROS is overwhelming, it will cause necrosis because of the irreversible degradation of cellular macromolecules (reviewed in [3]). When ROS cause damage to vital processes (e.g. DNA modification [19,20]), they can induce apoptosis [21–23]. However, when the increase in ROS is transient and moderate, it is not lethal, and the ROS may be detoxified within a few hours; hence the concept of 'oxidative stress'. However, the alteration in the antioxidant defences (especially glutathione regeneration), the recurrent uptake of pro-oxidant xenobiotics or chronic inflammation will lead to a more stable imbalance of the redox status.

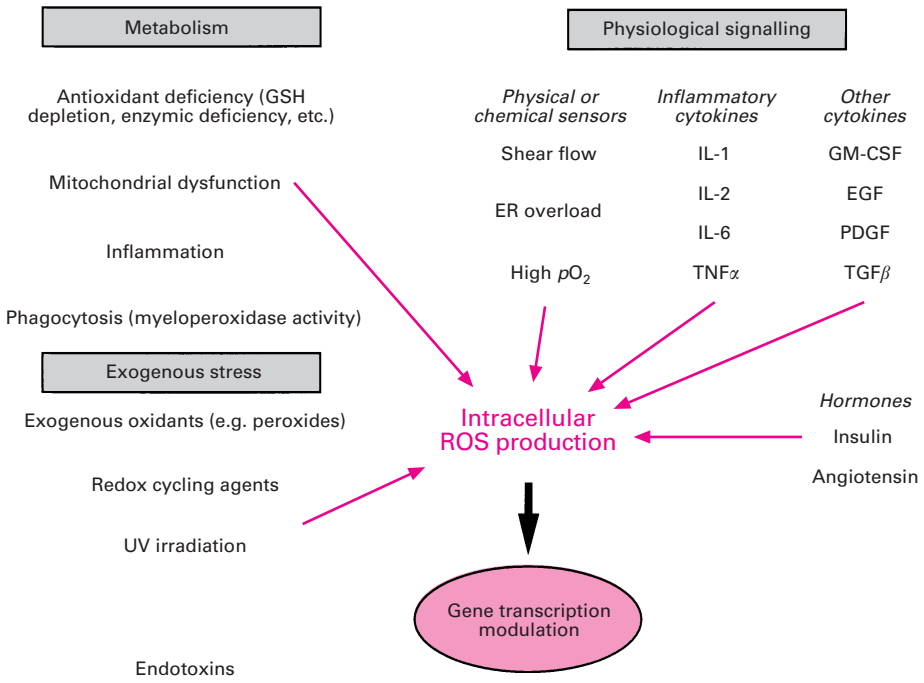


**Figure 2 Various factors elicit intracellular ROS production**

An exogenous stress, a metabolic dysfunction or a physiological stimulus can trigger ROS generation directly or indirectly. The resulting modulation of the redox status within the cell has an influence on the regulation of gene expression. Abbreviations: ER, endoplasmic reticulum; EGF, epidermal growth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; GM-CSF, granulocyte/macrophage colony-stimulating factor.

**Table 1 Genes that can undergo oxidative repression**

| Endogenous gene | Redox modulator/ROS generator | Reference(s) |
|---|---|---|
| IL-2 | $H_2O_2$ (extracellular) | 45 |
| | Xanthine oxidase activity | 52 |
| | Polyamine oxidase activity | 53 |
| TNFα (T cells) | $H_2O_2$ (extracellular) | 54 |
| CD3 (ζ chain) | $H_2O_2$ (extracellular) | 56 |
| CD16 (ζ chain) | $H_2O_2$ (macrophage-produced); diamide | 57 |
| Cyclins CLN1 and CLN2 (yeast) | $O_2$ (hyperoxia) | 70,71 |
| Glucokinase | $O_2$ (hyperoxia); $H_2O_2$ (extracellular) | 72 |
| PEPCK | $H_2O_2$ (extracellular) | 75 |
| Insulin | Glycation process | 73 |
| Tyrosine hydroxylase | $O_2$ (hyperoxia) | 85 |
| Tyrosine aminotransferase; tryptophan dioxygenase | Peroxidation products | 79 |
| pS2 | $H_2O_2$ (extracellular) | 84 |
| CYP1A1 | $H_2O_2$ (extracellular) | 92 |
| | Glutathione depletion; CYP1A1 activity | 93 |
| | TNFα; IL-1β | 94 |
| Several CYPs | Inflammatory cytokines | 86–88 |
| | Growth factors | 89,90 |
| Ferritin | $H_2O_2$ (extracellular) | 100 |
| | NO | 105 |
| EPO | $H_2O_2$ (extracellular) | 108,109 |
| | $O_2$ (normoxia and hyperoxia) | 110 |
| α-Actin; troponin I; myosin (light chain); creatine kinase (M isoform) | $H_2O_2$ (extracellular); glucose oxidase activity | 114 |
| Myosin creatinine phosphokinase (muscle) | NO; TNFα | 113 |
| Cytochrome *c* oxidase | $H_2O_2$ (extracellular); catalase knock-out | 64 |

## Oxidative stress, signalling and gene regulation

Some oxidation processes (such as cysteine oxidation) are reversible. They can therefore play a role in a dynamic regulatory process, as a result of a local or global variation in the redox conditions within the cell. Such a variation may cause a drastic modulation of the oxidized/reduced ratio of signalling proteins, such as transcription factors. Sublethal ROS production can thus interfere with signal transduction pathways [24–27]. As shown in Figure 2, ROS, in particular $H_2O_2$, are indeed second

messengers for various physiological stimuli, such as angiotensin [28], inflammatory cytokines and growth factors ([29] and references therein) or transforming factors [30]. In some cases, the activation of an NADPH oxidase by a ligand-stimulated membrane receptor complex was shown to result in generation of $H_2O_2$ within the cell [31]. Some stimuli have been shown to induce mitochondrial $H_2O_2$ release [21,32]. $H_2O_2$ is also generated by haemoproteins that are sensors of the oxygen tension [33,34]. Moreover, ROS are also produced as a result of a wide range of cellular stresses (e.g. shear flow [35], endoplasmic reticulum overload [36]).

One mechanism through which these effectors may elicit oxidative stress is the small G-protein Ras. Indeed, Ras is suspected to activate a cascade of kinases via ROS production [37]. It was also reported that Ras can activate the activator protein 1 (AP-1) and nuclear factor $\kappa$B (NF-$\kappa$B) responses without the intervention of kinases [38]. In addition, oxidative stress has been shown to activate several stress-activated protein kinases [extracellular-signal-regulated protein kinase, stress-activated protein kinase/c-Jun N-terminal kinase, p38 mitogen-activated protein kinase] or phosphatases [28,39–42]. In the case of a Ste20-like protein kinase, $H_2O_2$ appeared to be an exclusive activator [43]. Taken together, these observations suggest that ROS may mediate specific signalling pathways within the cell. Indeed, we will see below that proteins may be differently sensitive to oxidation according to their content of critical cysteine residues, to their conformation or to the intensity of the oxidative stress. Hence possible signal specificity may be mediated by oxidative stress.

Since these pathways lead to the modulation of gene expression, the role of oxidative stress as a modulator of transcription factors has been widely studied. Most studies have addressed the issue of transcriptional activation; in particular, activation of immediate-early genes by oxidative stress (e.g. genes encoding c-Jun [30,44,45], Egr-1 [44,46], Gadd153 [47], etc.). Some of these gene products (such as Jun, which is part of the AP-1 transcription factor), as well as other transactivators (such as NF-$\kappa$B), may in turn activate the transcription of detoxification enzymes [48] and antioxidant proteins, such as Trx [49,50]. However, even moderate ROS production can also specifically down-regulate the expression of various genes, which will now be discussed (see also Table 1).

## GENE REPRESSION BY OXIDATIVE STRESS

### Oxidative stress and alteration of the T cell response

Oxidative stress can repress the activity of T cells, and thus alter the immune response. A decrease in the GSH pool, which elicits oxidative stress by raising the intracellular redox potential (as described above), causes a marked inhibition of the T cell proliferative response [51]. Several laboratories have reported that oxidative stress inhibits interleukin 2 (IL-2) transcription. This cytokine is produced primarily by helper T cells, and regulates the growth and function of various cells involved in cellular and humoral immunity. Sublethal concentrations of $H_2O_2$ elicit a decrease in IL-2 mRNA levels. Moreover, they repress the transcription of a reporter gene driven by the IL-2 gene promoter, whereas they activate the promoter of the c-*jun* gene [45]. It was then shown that this repression is mediated by inhibition of the activity of a protein known as nuclear factor of activated T cells (or NFAT), through alteration of its binding to DNA. The intracellular generation of ROS by xanthine oxidase [52] or polyamine oxidase [53] has the same consequences. Thus, in contrast with the expression of several inflammatory cytokines (IL-1, IL-6, IL-8), IL-2 expression is decreased by oxidative stress. ROS appear to regulate differentially the various cytokines involved in immune functions. In T cells, the production of tumour necrosis factor $\alpha$ (TNF$\alpha$; an important inflammatory cytokine) is increased by various stimuli, such as phorbol esters. This stimulation is inhibited, at the transcriptional level, by micromolar concentrations of $H_2O_2$ [54]. However, this redox regulation of TNF$\alpha$ by oxidative stress depends on the cell type: in dendritic cells, TNF$\alpha$ expression is increased by $H_2O_2$ [55].

Oxidative stress also represses the gene encoding the $\zeta$ chain of CD3 and CD16 in blood peripheral lymphocytes and activated killer cells [56]. This regulation was observed in cultured T cells treated with $H_2O_2$ or diamide (a thiol-modifying reagent), and was prevented by raising the intracellular glutathione level. Moreover, T cells respond similarly when they are co-cultured with lipopolysaccharide-activated macrophages that release $H_2O_2$ [57]. This down-regulation of CD3 and CD16 may disrupt the T cell receptor complex and alter the immune response.

Mild but chronic oxidative stress (which is relevant in several pathophysiological processes) has the same repressing effect as described above. Indeed, during aging, ROS production is increased (see below). This redox imbalance modulates the expression of several genes involved in regulation of the immune system. On the one hand, activation of the transcription factor NF-$\kappa$B (see below) induces the expression of several inflammatory cytokines (IL-6, TNF$\alpha$). The resulting inflammatory signal elicits further ROS production, leading to a positive-feedback loop. On the other hand, the redox imbalance has been shown to cause a decrease in IL-2 expression in the T cells of rodents and humans. In this case also, a decrease in the activity of nuclear factor of activated T cells is involved in repression of the IL-2 gene promoter [58]. This could contribute to the alteration of the immune response in elderly or HIV-infected people. Patients suffering from rheumatoid arthritis (who have low intracellular GSH levels) display decreased IL-2 production, associated with hyporesponsiveness of synovial T cells to their specific stimuli [59].

### Oxidative stress and mitochondrial function

The mitochondrion is a one of the most powerful generators of ROS within the cell. In this organelle, the electron-deficient dioxygen molecule $O_2$ is brought close to electron suppliers. Indeed, the respiratory chain involves several successive complexes containing electron carriers (cytochrome *c*, ubiquinone, etc.) that allow the progressive and controlled reduction of $O_2$ into water. A dysfunction at one step of this chain may thus result in massive production of ROS. Typically, the inhibition of complex III leads to $H_2O_2$ release [60]. In addition, several stimuli, such as TNF$\alpha$ or ceramide, have been shown to induce mitochondrial $H_2O_2$ release [21,32].

The mitochondrion possesses a specific genome and produces its own RNAs that are necessary to its function. In mammalian cells, UVB radiation has been reported to repress mitochondrial function by strongly inhibiting mitochondrial transcription [61]. Crawford et al. [62] have shown that mitochondrial RNAs undergo specific degradation upon oxidative stress. Following treatment of hamster fibroblasts with $H_2O_2$, these authors observed that the 16 S rRNA, a major component of mitochondrial ribosomes, was specifically degraded, whereas cytosolic mRNAs were not. This resulted in a dramatic shut-down of mitochondrial protein biosynthesis. A similar observation was reported in human megakaryocytes, where endogenous $H_2O_2$ (produced as a result of homocysteine and $Cu^{2+}$ treatment) leads

to a decrease in mitochondrial RNA levels [63]. In addition, oxidative stress caused by $H_2O_2$ treatment, catalase knock-out or aging contributes to mitochondrial dysfunction in *Drosophila melanogaster* [64]. Oxidative stress was shown to reduce the levels of complex IV cytochrome *c* oxidase RNA.

The mitochondria seem to be very sensitive to oxidative stress. In hepatocytes from old rats, impaired mitochondrial function is associated with oxidative stress, and antioxidant supplementation leads to a recovery of this function [5]. In addition, glutathione depletion (causing intracellular ROS production) in neural cells was also shown to cause decreased mitochondrial function [65].

Mitochondria have been shown to be central integrators of apoptosis [23,66,67]. In addition, ROS seem to be involved in apoptosis (reviewed in [23]). It has been suggested that the global shut-down of mitochondrial function under conditions of oxidative stress could contribute to apoptosis because of the dramatic decrease in cellular energy supply.

Conversely, decreased mitochondrial activity under conditions of moderate oxidative stress limits further ROS release within the cell. This limitation of endogenous ROS production could be part of the adaptive response to oxidative stress (see Figure 6 below).

## Oxidative stress and growth arrest

Oxidative stress has a strong influence on the cell cycle. In several mammalian cell types, Wiese et al. [68] observed that micromolar $H_2O_2$ concentrations (that are non-apoptotic) induce temporary growth arrest and lengthening of the cell cycle. This slow-down of the cell cycle is correlated with the rapid *de novo* synthesis of at least 20 proteins. Another study reported that glutathione depletion in human cells also causes a $G_2$/M-phase arrest and delayed $G_1$- and S-phases, via a p53-independent mechanism [69].

In the yeast *Saccharomyces cerevisiae*, the mechanism of cell growth arrest under hyperoxia was investigated [70]. The arrest in $G_1$-phase was shown to be caused by inhibition of the Start function that prepares the yeast for S-phase. This deregulation results from inhibition of the transcription of two $G_1$-auto-regulated cyclins, CLN1 and CLN2. Unlike cyclin CLN3, which is constitutively expressed (under normoxic or hyperoxic conditions), CLN1 and CLN2 are inducible, and can trigger S-phase. Oxidative stress, which inhibits this induction, thus leads to transcriptional remodelling in eukaryotic cells. Another study in *S. cerevisiae* addressed the mechanism of $G_1$-phase lengthening. It reported that oxidative stress induced transcription of the XBP1 protein, a transcription factor that is related to cell cycle regulatory factors. The XBP1 gene promoter contains several stress-regulated elements, one of which responds positively to oxidative stress. XBP1 acts as a transcriptional repressor of a $G_1$ cyclin gene. Overexpression of XBP1 results in lengthening of $G_1$-phase and a slow-growth phenotype [71].

These observation clearly demonstrate that oxidative stress can specifically repress genes driving the cell cycle. The lengthening of $G_1$-phase allows time for the synthesis of enzymes that tend to buffer oxidative stress and can repair potential damage. This delay is important, since a base alteration could be converted into an irreversible mutation if a mismatch escaped the repair systems before replication. Hence there is a necessity not to activate S-phase too quickly when a cellular stress occurs. A cell cycle arrest is required in order to assess the amount of macromolecule alterations, and, if necessary, to enter the apoptotic pathway instead of carrying on the cellular division process.

## Redox modulation of carbohydrate metabolism

In the liver acinus, glucose is produced in the periportal region and degraded in the perivenous region. This is correlated with the periportal location of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and the perivenous location of the glycolytic enzyme glucokinase. The opposing regulation of the expression of two key enzymes in carbohydrate metabolism is correlated with differential $O_2$ pressures [33]. Oxygen indeed plays a major role in the zonation of carbohydrate metabolism and associated gene expression in the liver. Hepatocytes located around the afferent (periportal) or efferent (perivenous) vessels are irrigated by blood which contains respectively high or low dioxygen concentrations. It is thus likely that their intracellular redox status differs. It has been reported that the induction of glucokinase by insulin is repressed by high (i.e. arterial) $O_2$ pressure [72], whereas the induction of PEPCK by glucagon is repressed by low (i.e. venous) $O_2$ pressure. In cultured hepatocytes, $H_2O_2$ mimics arterial $O_2$ for the repression of glucokinase expression [72]. $H_2O_2$ is thus likely to be a relevant marker of $O_2$ pressure. It has been proposed that a system involving a haemoprotein able to produce $H_2O_2$ could act as a sensor of $O_2$. $H_2O_2$ production by hepatocytes has indeed been observed as a function of $O_2$ tension [33].

Oxidative stress may regulate carbohydrate metabolism through other pathways. For example, the production of ROS associated with the glycation process was reported to inhibit insulin mRNA production in pancreatic $\beta$ cells [73]. This down-regulation is consistent with the observation that diabetes mellitus is associated with pancreatic oxidative stress. Moreover, catalase gene transfer in human pancreatic islet cells allows unaltered insulin production under conditions of oxidative stress [74]. Taken together, these observations show that oxidative stress can negatively modulate the expression of genes that control carbohydrate metabolism by repressing the insulin signalling pathway. The down-regulation of insulin expression by oxidative stress is consistent with this observation: insulin is a repressor of PEPCK and an inducer of the glucokinase gene. Thus oxidative-stress-based gene regulation may be integrated in the physiological regulation of carbohydrate metabolism.

However, the complex mechanisms controlling the latter function are still unresolved. For example, $H_2O_2$ has been classically described as an insulino-mimetic agent. The group of Granner [75] has reported that $H_2O_2$ could repress both the endogenous and the transfected PEPCK gene, by an as yet unknown mechanism that does not involve either p38 mitogen-activated protein kinase (a kinase typically activated by $H_2O_2$) or phosphoinositide 3-kinase. These discrepancies are still unexplained, but could be related to differences in the amount of $H_2O_2$ added to the cells, and thus to different levels of oxidative stress.

## Redox modulation of hormonal responses

Several studies reported by different groups have consistently shown that oxidative stress can alter hormonal regulation. It was first reported that oxidation *in vitro* by tetrathionate of the glucocorticoid receptor (GR) resulted in the formation of a disulphide bridge within the protein [76]. This oxidative inhibition of the GR was reversed by the thiol-reducing agent dithiothreitol. $H_2O_2$ was then shown to induce the formation of a disulphide bond between two close cysteine residues located within the GR DNA-binding domain (DBD) [77]. Evidence for the redox modulation of glucocorticoid function in cell cultures has also been obtained. In COS cells, glutathione depletion or $H_2O_2$ treatment altered the DNA binding of the GR [while that of

another transcription factor, CAAT enhancer binding protein (or C/EBP), was unaffected]. This effect was prevented by the glutathione precursor *N*-acetylcysteine. Furthermore, the induction by dexamethasone (a synthetic glucocorticoid agonist) of reporter gene expression driven by glucocorticoid-responsive elements was repressed by GSH depletion [78]. The redox modulation of the GR response is relevant *in vivo*. Indeed, rats treated with compounds derived from linoleic acid (resulting in endogenous hepatic oxidative stress) displayed an altered hormonal response. Under these conditions, the tyrosine aminotransferase and tryptophan 2,3-dioxygenase genes exhibited a low response to glucocorticoids. In contrast, the insulin and glucagon responses were not affected by moderate oxidative stress [79].

Makino and colleagues [49] have extensively studied the role of redox modulation in hormonal regulation. Initially they showed, using transfection experiments, that antisense Trx mRNA expression mimicked the repressive effect of $H_2O_2$ on genes driven by glucocorticoid-responsive elements. The impaired cellular response to glucocorticoids was rescued by Trx overexpression. This suggests that cellular glucocorticoid responsiveness is coordinately modulated by the redox state. In CHO cells expressing the human GR, the same group showed that $H_2O_2$ treatment decreased the ligand-binding and transcriptional activity of this receptor. They also confirmed that the DBD was repressed by thiol-oxidizing reagents. This repression was prevented by a novel antioxidant named EPC-K1 [80]. Recently, these workers produced evidence that an association between Trx and the GR DBD occurs in the nucleus to restore GR function under conditions of oxidative stress. They suggested that Trx, via a direct association with the conserved DNA-binding motif, may represent a key mediator operating in the interplay between cellular redox signalling and nuclear-receptor-mediated signal transduction [81]. In addition, the same group showed that oxidative conditions impaired both ligand-dependent and -independent nuclear translocation of the GR. Cys-481, located in the nuclear localization signal domain NL1 of the GR, is critical for this regulation [82]. Its substitution with serine abolishes the redox regulation of nuclear translocation.

Convincing, although less extensive, evidence has shown that oestrogen receptor (ER) function is likewise repressed by oxidative stress [83,84]. In human breast cancer cells, micromolar $H_2O_2$ concentrations inhibit pS2 mRNA production. The *pS2* gene is a well known target of the ER in mammalian cells. In transfection experiments, ER-driven reporter genes were also repressed by oxidative stress. Overexpression of Trx can prevent this effect. As with the GR, transfection of expression plasmids for antisense Trx mRNA also decreased expression of an ER-responsive reporter gene. Moreover, the specific DNA binding of recombinant ERs was inhibited by thiol-modifying agents, and this effect could be reversed by Trx addition. The modification of cysteine residues within the DBD of the ER was shown to induce a conformational change (loss of $\alpha$-helix structure). Since oxidative stress does not affect the cellular content of ER or its ligand-binding capacity, the DBD seems to be the main target of ER redox regulation [83].

In addition, it was reported that, in an adrenal-gland-derived cell line, intracellular $H_2O_2$ generation (probably by a haemoprotein sensor) following hyperoxia repressed the expression of the tyrosine hydroxylase gene [85]. Since this enzyme is rate limiting in the synthesis of catecholamines, this redox regulation could influence the secretion of these hormones.

Taken together, these observations show that cellular glucocorticoid, oestrogen and possibly catecholamine signalling is coordinately modulated by the redox state. In this respect, endogenous redox modulators such as Trx play a major role (see below).

### Repression of *CYP* genes by oxidative stress

The CYPs are a superfamily of ubiquitous enzymes involved in the metabolism of a wide range of either endogenous or exogenous (xenobiotic) compounds. These mono-oxygenases have been studied intensively because of their important functions in drug and pollutant metabolism. Some isoforms are particularly inducible at the transcriptional level by specific compounds. In addition to these specific regulations, several *CYP* gene promoters have been shown to be regulated by physiological signals, such as glucocorticoids, inflammatory cytokines and growth factors (reviewed in [86]). Morgan and colleagues ([87,88] and references therein) showed that several inflammatory cytokines (IL-1, IL-6, IL-11), interferon inducers and various growth factors (epidermal growth factor, transforming growth factors $\alpha$ and $\beta$, hepatocyte growth factor) down-regulated expression of the *CYP2C11* gene and other liver-expressed isoforms. Other groups showed that cytokines such as TNF$\alpha$ and IL-1$\beta$ repressed the expression of CYPs belonging to subfamilies 1A, 3A, 2B and 2E [89,90]. Moreover, expression of major liver CYPs (belonging to the 2E, 2C and 3A families) was shown to be repressed by endotoxins in the acute-phase response to lipopolysaccharide. This inhibition was not mediated by NO, a known mediator of the decrease in the catalytic activity and expression of several CYP isoforms [91].

Since ROS have been shown to be second messengers of several physiological signals (see the Introduction section) that are implicated in *CYP* gene regulation, the effect of oxidative stress was investigated. CYP1A1 is an isoform that is highly induced by planar aromatic compounds (such as dioxin or benzo[*a*]pyrene) and which is able to metabolize a wide range of substrates, in particular polycyclic aromatic hydrocarbons. We and others observed that, in hepatoma cells treated with dioxin, CYP1A1 expression was greatly decreased by oxidative stress ($H_2O_2$ treatment [92,93] or glutathione depletion [93]). In addition, IL-1$\beta$ [94] and TNF$\alpha$ [90,93] were shown to inhibit the expression of this CYP isoform. For TNF$\alpha$, this effect was shown to be mediated by ROS [93]. This ROS-mediated repression was, in turn, shown to be mediated by a nuclear factor I (NFI) site located in the promoter [93]. We showed that the transcription factor NFI was particularly sensitive to oxidative stress (see below).

Interestingly, several CYP isoforms have been shown to release ROS during their catalytic cycles, especially with uncoupled substrates ([95,95a]; and see Introduction section). This ROS production could contribute to repress the expression of oxidative-stress-sensitive genes. A possible consequence could be a negative-feedback mechanism controlling *CYP* gene expression. Indeed, high CYP1A1 activity within the cell represses the promoter of its own gene [96,96a]. This negative autoregulation could limit the intracellular production of ROS by CYP1A1 (and subsequent damage, such as DNA alterations [97]), as well as the activation of particular CYP1A1 substrates into carcinogenic compounds (e.g. benzo[*a*]pyrene). We are currently investigating the autoregulatory mechanisms of some CYP isoforms. The negative regulation of transcription by ROS could affect several CYP isoforms. Indeed, the intrahepatic zonation of several CYP isoforms is not homogeneous (reviewed in [98]). In the periportal region, where oxygen partial pressure ($pO_2$) is relatively high, CYP mRNAs are less abundant than in the perivenous region, where $pO_2$ is low. In addition, a high $pO_2$ contributes to ROS generation, either by increasing the probability of uncontrolled

partial reduction of oxygen or by stimulating $pO_2$ sensors (haemoproteins) which can release $H_2O_2$. Indeed, intrahepatic $H_2O_2$ production was observed to be a function of oxygen tension [33].

The repression of enzymes that release ROS could be part of a concerted stress response. Indeed, under conditions of oxidative stress (i.e. ROS production within the cell that exceeds its antioxidant capacity), it is important not only to induce ROS scavengers but also to repress ROS generators. Thus the cellular response to oxidative stress consists of opposite but complementary transcriptional regulation: the induction of antioxidant enzymes and the inhibition of putative ROS-releasing enzymes such as mono-oxygenases (see Figure 6).

### Iron metabolism

The iron-regulatory proteins IRP-1 and IRP-2 play an important role in the expression of genes required for iron metabolism. These proteins can bind to a specific mRNA sequence known as the iron-responsive element (IRE). IRP-1 contains an [Fe–S] cluster which is important for the regulation of its activity. At least six mRNAs are regulated by an IRE sequence (for a review, see [99]). The binding of IRP-1 to the IRE of the transferrin receptor mRNA leads to stabilization of this mRNA and increased expression of this receptor. In contrast, when IRP-1 binds to the mRNA coding for ferritin, translation is repressed. IRP-1 activity is up-regulated by iron deficiency and repressed when iron is in excess, in which case sequestration prevails upon uptake. Various modifications of the IRP proteins modulate their RNA binding, among which oxidoreduction plays a major role. The mechanism of regulation of IRP-1 by oxidative stress is still unclear. Several studies have reported that the oxidation of IRP-1 in a cell-free system, using xanthine oxidase as a generator of ROS, decreases its activity [100]. This inhibition is prevented or reversed by the antioxidant enzymes SOD and catalase, as well as by $\beta$-mercaptoethanol (a disulphide reducing agent) or *N*-acetylcysteine (a glutathione precursor). Thus IRP-1 appears to be a direct target of ROS. Indeed, in a study of the effect of the strong biological oxidant peroxynitrite on IRP-1 activity, Drapier and colleagues [101] found that Cys-447 was critical. Its mutation abolished the oxidation of human recombinant IRP-1 by peroxynitrite. It was thus postulated that oxidants could promote disulphide-bridge formation in the vicinity of the IRE-binding domain. In the same laboratory, Trx (an intracellular reducing agent) was found to activate IRP-1 and to restore the spontaneous IRE binding of IRP-2 which was altered by NO. In contrast with these observations, Pantopoulos et al. [102] have shown that extracellular addition of micromolar $H_2O_2$ rapidly activated the binding of IRP-1 to the IRE through an unknown signalling pathway. However, under the same conditions, intracellular $H_2O_2$ production does not activate IRP-1 activity. These different modes of regulation are discussed below.

The modulation of iron metabolism by the NO radical is complex. The generation of NO following interferon-$\gamma$/lipopolysaccharide treatment of macrophages can both slightly activate IRP-1 and strongly repress IRP-2 [103,104]. The same stimulus was reported to activate ferritin expression [103] and to reduce the level of transferrin receptor mRNA [105]. In this respect, NO seems to act like intracellular ROS, as described above. IRP-1 contains an [Fe–S] cluster that was suggested to be targeted by NO. In contrast, IRP-2 does not contain such a cluster, which may explain its opposite regulation by NO.

Two physiological processes may account for the complex redox regulation of IRP activity. First, in order to explain the activation of IRP-1 by micromolar extracellular $H_2O_2$, it has been proposed that this molecule could play a role as a mitogenic signal, whereby the activation of a receptor and a specific signal transduction pathway may be involved. Therefore cellular proliferation, which requires the synthesis of additional Fe-containing proteins, could explain the increase in iron uptake. Concerning the actual redox modulation of IRP within the cell, oxidants such as $H_2O_2$, $O_2^{-\bullet}$ or peroxynitrite are inhibitors. With intracellular ROS production, the fact that iron sequestration prevails upon uptake appears to be a natural defence mechanism. This will limit free iron and thus limit the deleterious Fenton reaction (which produces the hyper-reactive hydroxyl radical $OH^{\bullet}$) and further spreading of the oxidative stress. To allow this sequestration, direct oxidation of IRP inhibits IRP–IRE binding, so that the expression of ferritin is increased, while that of the transferrin receptor is repressed. Consistent with this, hypoxia (i.e. low $O_2$) regulates IRP activity in an opposite manner. Indeed, hypoxia induces the transcription factor hypoxia-inducible factor-1$\alpha$ (HIF-1$\alpha$), which activates the erythropoietin (EPO) gene promoter. EPO stimulates erythroid proliferation and concomitant haemoglobin synthesis. EPO was shown to activate IRP-1–IRE binding activity in order to stimulate iron uptake [106]. ROS prevent this signalling pathway, because oxidative stress is a repressor of HIF-1$\alpha$ (see below) and of EPO synthesis. This physiological signalling pathway is consistent with the inhibition of IRP activity by oxidative stress, as mentioned above. Moreover, in fibroblasts, the inflammatory cytokine TNF$\alpha$ (which elicits ROS production within the cell; see the Introduction section) stimulates ferritin transcription (i.e. increases iron sequestration). The mechanism seems to involve a transcription factor of the NF-$\kappa$B family which is activated by ROS [107]. Conversely, anti-inflammatory cytokines such as IL-4 and IL-13 were reported to enhance iron uptake in macrophages [105].

### EPO expression

EPO, a hormone that is mainly produced in the kidneys, stimulates erythroid proliferation. In this respect, it is involved in iron metabolism (as mentioned above). Fandrey and colleagues [108,109] reported that, in several models (hepatoma cell lines, perfused kidneys), EPO expression is repressed by ROS (mainly $H_2O_2$). It was also reported that EPO mRNA levels are decreased by $H_2O_2$ [110]. The full expression of EPO is allowed under conditions of low $pO_2$ through the activation of the gene promoter by HIF-1$\alpha$. This transcription factor is inactivated under conditions of normal $pO_2$ (normoxia). Its regulation will be described below. The ROS produced under normoxic conditions, and to a greater extent under hyperoxic conditions (i.e. oxidative stress), could mediate EPO gene repression. It has been suggested that a cytochrome-like haemoprotein could play the role of oxygen sensor, and release $H_2O_2$ [34]. Indeed, treatment of normoxic cells with exogenous catalase stimulates EPO production [111].

Interestingly, the down-regulation of HIF-1$\alpha$ by oxidative stress could also be part of a negative-feedback mechanism. Indeed, it has been reported that two stimuli that induce the EPO gene (hypoxia and cobalt chloride) lead to mitochondrial ROS generation [112]. These ROS could, in turn, repress HIF-1$\alpha$ (by increasing its degradation) and thus inhibit expression of HIF-1$\alpha$-driven genes (such as the EPO gene).

### Muscle genes

Oxidative stress has been suggested to be responsible for the decreased body weight, muscle wasting and skeletal-muscle

molecular abnormalities of cachexia observed in TNF$\alpha$-treated mice [113]. In cardiac muscle cells, ROS production elicited by TNF$\alpha$ or activation of NO synthase decreases the expression of myosin creatinine phosphokinase. The treatment of mice with antioxidants prevented these abnormalities. Another study showed that oxidative stress specifically repressed cardiac muscle genes [114]. In cardiocyte cultures treated with exogenous $H_2O_2$ or with glucose/glucose oxidase (which generates $H_2O_2$), the mRNA levels of the muscle-specific genes cardiac $\alpha$-actin, troponin I, myosin light chain 2 and the M isoform of creatine kinase were decreased, whereas those of the non-muscle genes pyruvate kinase and $\beta$-actin were unaffected.

## OXIDATIVE STRESS AND TRANSCRIPTION FACTORS

### Molecular insights

The mechanisms controlling the induction of genes by oxidative stress have been intensively investigated. It has been shown that the transcription factors NF-$\kappa$B and AP-1, which are stimulated by ROS [115], could mediate such inductions (for reviews, see [1,116,117]). The antioxidant-responsive element sequence, which can bind several proteins, plays a important role in gene promoters induced by oxidative stress (for a review, see [118]). One of these proteins, Nrf2, has recently been shown to be derepressed by oxidative stress [119]. Thus ROS were found to be able to interfere with gene expression at the transcriptional level. Proteins are susceptible to modification by oxidation, mainly on sulphur-containing residues (cysteines, but also methionines [120]). The oxidation of such residues is easily observed *in vitro*, but the challenge is to establish the functional relevance of these alterations *in vivo*. Numerous studies have shown that a variation of the redox status within the cell can alter the function of transcription factors. This redox change can target transcription factors directly or can be mediated by other signals, such as phosphorylation/dephosphorylation or glycosylation. The redox regulation of transcription is now well established. For example, in *Escherichia coli*, the transcription factors SoxR and OxyR have been clearly shown to be activated by the oxidation of a [2Fe–2S] cluster, and are thus $O_2^{-\bullet}$ and $H_2O_2$ sensors respectively.

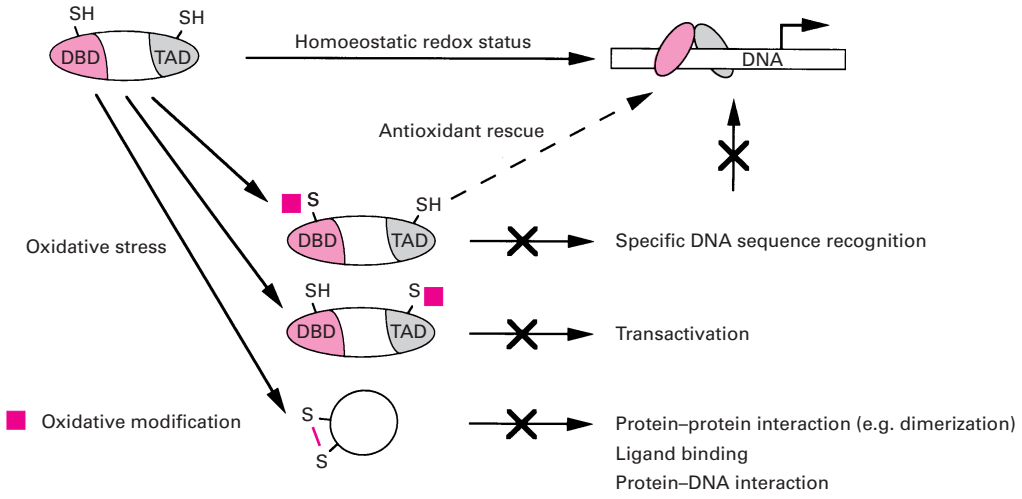


**Figure 3 Oxidative inhibition of a transcription factor**

When a critical cysteine residue undergoes an oxidizing modification, the function of a protein can be dramatically altered. In transcription factors, critical cysteines may be found within the DBD. Their oxidative modification (indicated by the red squares) can impair direct protein–DNA interactions. The uncontrolled formation or disruption of a disulphide bridge can alter the global conformation of the protein, which may alter important activities, such as dimerization, ligand binding, and protein–protein or protein–DNA interactions. In a transcription factor, a cysteine residue may be located in the TAD. Its oxidation, even if it does not alter DNA-binding activity, may affect crucial interactions with other transcription factors, co-activators or the transcription machinery, and blunt the transactivation process.

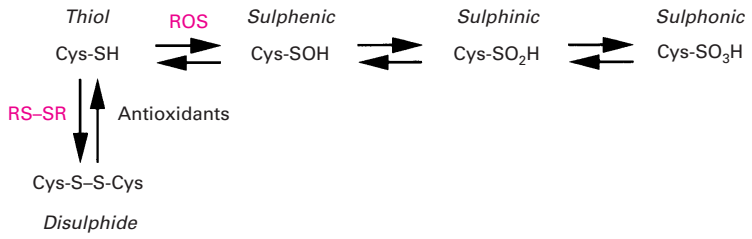


**Figure 4 Oxidative modification of a cysteine thiol moiety**

Cysteine residues may undergo chemical modifications on their thiol -SH moieties. The sulphur atom may be oxidized by ROS or a compound containing a disulphide bridge (RS–SR), such as GSSG. The S–H covalent bond is then replaced by an S–O or an S–S covalent bond to yield sulphenic, sulphinic or sulphonic groups, or a disulphide bridge respectively. These modifications are reversible by antioxidant reagents containing a reduced thiol group, such as dithiothreitol, $\beta$-mercaptoethanol, *N*-acetylcysteine or glutathione.

**Table 2 Transcription factors that can undergo oxidative repression**

Abbreviations: SV40, simian virus 40; NLS, nuclear localization signal; PEBP2/CBF, polyoma virus enhancer-binding protein 2/core binding factor.

| Transcription factor | ROS target | Related gene redox regulation | Reference(s) |
|---|---|---|---|
| Sp1 | DBD ($Cys_2His_2$ zinc fingers) | SV40 (viral promoter); $\beta$-enolase; dihydrofolate reductase | 129–132 |
| NFI | Several cysteines within the DBD | CYP1A1 | 126 |
| | A cysteine within the TAD | CYP1A1 | 93 |
| GR | Cysteines within the DBD | Tyrosine aminotransferase | 76–78 |
| | Cys-481 within the NLS | Tryptophan dioxygenase | 49,82 |
| ER | Cysteines within the DBD | pS2 | 83,84 |
| USF | Cys-229 and Cys-248 (DBD) | – | 135 |
| MyoD | Cys-135 | – | 137 |
| HIF-1$\alpha$ | Cys-774 (TAD) | EPO | 139,141,143,144 |
| PEBP2/CBF | Cys-124 (DBD) | – | 159 |
| AP-1 (Jun) | Cys-252 (DBD) | – | 160 |
| AP-1 (Fos) | Cys-154 (DBD) | – | 160 |
| NF-$\kappa$B (p50) | Cys-61 (DBD) | – | 167,168 |
| p53 | Several cysteines within the DBD | – | 148,149,151,152 |

In the reduced state SoxR still binds to DNA, but it does not activate transcription [121]. The redox potentials that control the activity of these factors have been determined [122,123].

A large number of eukaryotic transcription factors are sensitive to modulation of the redox status within the cell. Their regulation may be positive or negative but, so far, owing to the historical focus on gene induction, the former situation has been most studied (for reviews, see [116,117]). The sensitivity of a transcription factor is variable, and depends essentially on its conformation and cysteine content. Most transcription factors contain strategic cysteine residues. Those located in the DBD may be crucial for DNA site recognition, where the thiol groups can interact with bases via hydrogen bonds or electronic interactions. Cysteine residues may be located elsewhere and make a critical contribution to the global conformation of the protein because of the formation of disulphide bridges or metal ion chelation (e.g. zinc-finger proteins). The oxidation of a cysteine may result in a functional alteration of the protein (Figure 3). The abnormal formation of a disulphide bridge can modify protein conformation and abolish dimerization (frequently required for transcription factor activation) or DNA recognition processes. An abnormal redox environment may also disrupt a useful disulphide bridge and lead to the formation of another one (involving the same cysteine residue). The cysteine residues may also undergo oxidation of their thiol moieties without necessarily forming a disulphide bridge (Figure 4). The thiol group (-SH) can gain oxygen atoms to yield sulphenic (-SOH), sulphinic ($-SO_2H$) or sulphonic ($-SO_3H$) moieties [124]. In this case, the electronic and steric conformation of the cysteine residue can be drastically modified. This can, in turn, alter the function of a transcription factor if this cysteine is critical for a protein–protein or protein–DNA interaction. We describe below a representative list of transcription factors whose function is impaired by oxidative stress (see also Table 2).

## Inhibition of transcription factor activity by oxidative stress

The regulation of a gene is controlled by the activity of several transcription factors. These proteins act in co-operation with each other in a complex network involving both protein–DNA and protein–protein interactions. If a precise transcription factor plays a predominant role in the transactivation of a gene promoter, its inhibition by redox modification can result in a dramatic decrease in promoter activity and subsequent gene expression.

### NFI

NFI was first described as an activator of DNA replication. It also appeared to be a member of a family of ubiquitous transcription factors binding to the palindromic TTGG-$CN_5$GCCAA consensus sequence or to a simple half-site [125]. Four genes code for the various isoforms, which can combine in homo- and hetero-dimers. The redox regulation of NFI is especially interesting, as oxidative stress affects both its DNA-binding and transactivating functions. The highly conserved DBD of 220 amino acids is not similar to any well characterized class of DNA-binding molecules. It contains four cysteines, three of which are required for the DNA-binding activity. Their mutation to serine residues or their *in vitro* oxidation abolishes this activity [126]. Moreover, glutaredoxin (a thioltransferase that can reduce oxidized thiol moieties) increases NFI DNA binding ([127], and references therein). In addition, NFI DNA-binding activity was shown to be altered within the cell by $H_2O_2$ treatment or glutathione depletion [93]. As a negative control, we showed that, under the same conditions, the ubiquitous CCAAT-binding protein 1 (CP-1) transcription factor remained unaltered. This differential regulation of NFI and CP-1 seems to be involved in a redox switch when these two transcription factors compete for the same DNA promoter sequence (see below). Moreover, the expression of reporter genes driven by NFI is strongly impaired by oxidative stress ($H_2O_2$ treatment or glutathione depletion). Interestingly, the repression of NFI transcriptional activity by $H_2O_2$ was particularly potent, and was observed at concentrations that do not affect DNA binding. Using Gal4 fusion proteins, we observed that the transactivating domain (TAD) of NFI/CCAAT transcription factor, which contains two cysteines, was particularly sensitive to $H_2O_2$ [96a]. We are currently investigating the amino acid target of oxidative stress. Our results suggest that a cysteine located within the TAD is required to mediate the repressive effect of $H_2O_2$ (Y. Morel, N. Mermod and R. Barouki, unpublished work). The regulation of TADs by micromolar concentrations of ROS could be a novel mechanism involved in the regulation of genes by oxidative stress. So far, most of these regulatory mechanisms, at least in mammalian cells, have been explained by oxidative alteration of the transcription factor DBDs.

### Sp1

The Sp1 transcription factor is ubiquitous and binds to GC-rich DNA sequences present in a wide range of promoters, particularly in those that do not contain a TATA box motif [128]. The Sp1 protein contains three zinc-finger motifs that are crucial for DNA-binding activity. Sp1 DNA binding was shown to be particularly sensitive to thiol-oxidizing or -alkylating reagents *in vitro* (while other factors, such as nuclear factor-Y and high-mobility-group proteins, were unaffected) [129]. Zinc co-ordination as well as DNA binding appear to protect Sp1 from oxidative stress [130]. The DNA-binding activity is impaired *in vivo* by $H_2O_2$ and thiol-modifying reagents [131]. This alteration is reversible, since nuclear extracts of $H_2O_2$-treated cells can recover DNA-binding activity after treatment with dithiothreitol (a thiol-reducing compound). Moreover, it has been reported that low GSH concentrations within the cell also decreased Sp1 DNA binding, whereas the transcription factor CAAT enhancer binding protein (or C/EBP) remained unaffected [78,130]. The transcriptional activity of Sp1 has also been shown to be altered *in vivo* by oxidative stress. Sp1-driven genes, such as aldolase A, pyruvate kinase M2, $\beta$-enolase and dihydrofolate reductase, were repressed by $H_2O_2$ treatment [131,132]. Under the same conditions, the haem oxygenase and metallothionein genes were activated.

One consequence of the regulation of Sp1 by oxidative stress is that viral promoters and enhancers that include Sp1 sites (such as simian virus 40) will be regulated. Thus one should be cautious when these promoters are used as transfection assay controls in studies of gene regulation by oxidative stress. Consistent with the results obtained in cell cultures with exogenous oxidants, HIV-infected cells display altered Sp1 activity [133]. Indeed, the HIV Tat protein represses the SOD gene, and thus HIV-infected cells display chronic oxidative stress. Furthermore, a chronic shift in the intracellular redox status to more oxidant conditions was observed in aged rats. This was also associated with decreased Sp1 DNA-binding activity [134]. Taken together, these observations suggest that the Sp1 transcription factor is a sensitive target of oxidative stress *in vivo*. Since Sp1 is a ubiquitous transcription factor, it appears to be a major mediator of gene repression by redox modulation within the cell.

### Transcription factors containing a helix–loop–helix (HLH) motif

Several transcription factors contain a HLH motif. They bind as dimers to the CANNTG sequence (called the E box) in numerous gene promoters. The function of several such transcription factors is altered by oxidative stress, as described below.

The upstream stimulatory factor (USF) transcription factor contains only two cysteine residues, which are located at amino acids 229 and 249 within the HLH motif. The latter is required for the dimerization of USF. The mutation of these cysteines to serines does not affect DNA-binding activity. Oxidative conditions lead to the formation of abnormal intra- or intermolecular disulphide bridges. This inhibits the dimerization process and thus prevents the binding of USF to its target DNA sequence [135]. In contrast, the thiol-reducing reagent dithiothreitol increases USF DNA binding to the E box motif. Thus, in the case of USF, the two cysteines are not required for DNA binding, but they act as sensors of oxidative stress by repressing the activity of this transcription factor. The serine mutants are not sensitive to oxidative stress. Furthermore, the promoter of the insulin gene, which is activated by USF and several other related factors [136], has been shown to be repressed by oxidative stress [73].

MyoD is a HLH transcription factor important for muscle cell growth and differentiation. It undergoes a conformational change upon oxidation that strongly decreases its specific DNA binding. It has been shown that the mechanism involves the oxidation of Cys-135 [137].

Another interesting example is HIF-1$\alpha$. This transcription factor is involved in the regulation of the EPO gene, as described above. Considerable work has recently been undertaken in order to understand its regulation. This protein contains a bHLH–PAS (basic HLH–Per-Arnt-Sim) domain. This is a conserved sequence structure [138] found in several regulatory proteins, such as the aryl hydrocarbon receptor (AhR) and the AhR nuclear translocator (Arnt) (which constitute the dioxin receptor). The C-terminal part of HIF-1$\alpha$ contains two transactivating domains, named NAD and CAD [139,140]. HIF-1$\alpha$ is constitutively expressed, but is rapidly degraded under normoxic and oxidative conditions [141]. It has been shown that normoxic oxygen tension induces the degradation of an oxygen-dependent degradation domain (which overlaps the NAD) by the ubiquitin/proteasome pathway [142]. Conversely, HIF-1$\alpha$ is stabilized by hypoxia and by antioxidants through an as yet unknown mechanism ([143], and references therein) and then heterodimerizes with HIF-1$\beta$ (more commonly known as Arnt) to transactivate gene expression. It was proposed that a haemoprotein system is part of the cellular oxygen sensor system leading to HIF-1$\alpha$ degradation [144]. Apart from protein stabilization/degradation, HIF-1$\alpha$ undergoes another redox regulation. It has been recently reported that the CAD transactivating domain could interact with the co-activator p300/CREB-binding protein [139]. Ref-1 and Trx enhance this transactivating interaction. Furthermore, a critical cysteine residue (Cys-774) is required for this interaction. It was shown that its reduction by endogenous redox-active proteins (such as Ref-1 or Trx) is critical in mediating the transactivating function of HIF-1$\alpha$.

The regulation of the AhR [which binds to so-called xenobiotic-responsive element (XRE) DNA sequences] by oxidative stress is unclear. Its DNA binding is not affected by oxidative stress [145]. Transfection studies on the induction of the *CYP1A1* gene showed that the response mediated by XRE sequences seemed to be unaffected by $H_2O_2$ concentrations that strongly repress NFI [93]. However, another study showed that higher (millimolar) $H_2O_2$ concentrations could repress reporter genes driven by XRE sequences [145]. Thus the AhR may be sensitive to strong oxidation conditions but remain unaffected by moderate ROS concentrations.

### p53

The well-known tumour-suppressor protein p53 has been shown to be a transcription factor [146]. It contains 12 cysteine residues in its amino acid sequence [147], nine of which are located within the DBD. Four of the latter are required for DNA-binding activity (mutation of the others to serine does not alter binding activity). Moreover, three cysteines are involved in zinc co-ordination. *In vitro* oxidation leads to several non-functional p53 conformations (DNA-binding impairment [148]). In addition, the *in vivo* DNA binding of p53 is impaired by $H_2O_2$, with the level of the p53 protein being unaltered. The expression of a reporter gene driven by a p53-responsive promoter is also decreased by oxidative treatment [149]. Furthermore, the conformation of p53 is sensitive to metal cations. Redox modulation within the cell caused by copper uptake and $Cu^{2+}/Cu^{+}$ redox cycling also inhibits p53 DNA binding *in vivo* by a mechanism that does not involve ROS production [150]. The perturbation of the intracellular copper content by agents such as pyrrolidine

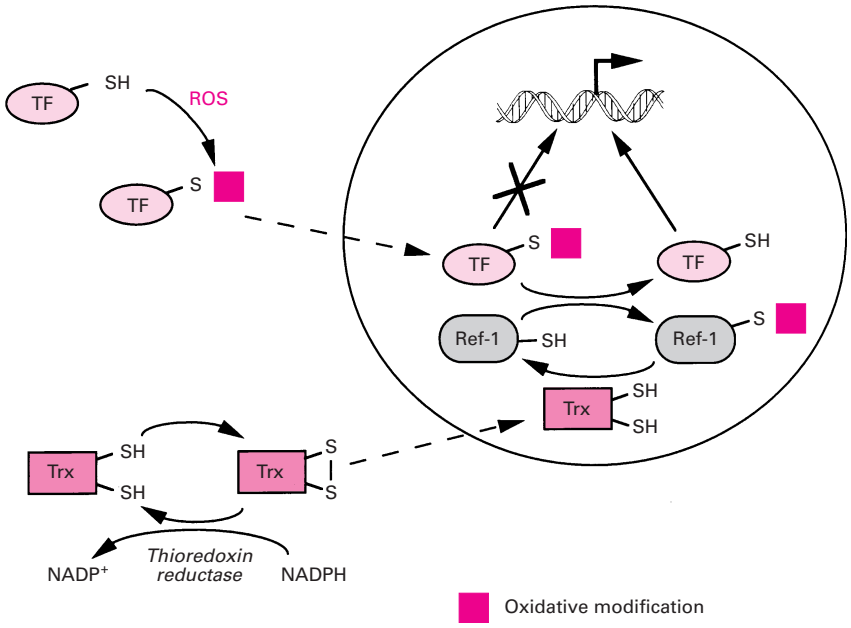


**Figure 5 Rescue mechanism to re-activate oxidized transcription factors**

The Trx molecule acts like glutathione and can reduce oxidized thiol groups. Oxidized Trx is regenerated by Trx reductase. Under conditions of oxidative stress, Trx is translocated into the nucleus, where it can interact directly with a transcription factor (TF) or be involved in an oxidation/reduction cascade comprising Ref-1. This mechanisms allow a maximal efficiency of several transcription factors to mediate gene transcription.

dithiocarbamate (which binds and transports extracellular copper) thus prevents p53 activation by known stimuli (UV, temperature shift). This inhibition of p53 function was shown to be caused by cysteine oxidation *in vivo* [151]. Furthermore, the redox modulator Ref-1 (see below) was reported to re-activate oxidized p53 and stimulate p53 transactivating function *in vivo* [152].

Thus it is well established that oxidative conditions alter the activity of p53 within the cell and related gene expression. This inhibition of a tumour suppressor may be correlated with the observation that abnormal cellular oxidative stress is associated with carcinogenesis. Conversely, since p53 is known to induce apoptosis by eliciting mitochondrial ROS production [66], the repression of p53 activity could allow the cell to escape from the p53-mediated apoptotic pathway in cases of transient intracellular ROS production.

## The crucial role of ubiquitous redox factors

As described above, the DNA-binding activity of transcription factors often involves critical cysteine residues. This activity can be impaired if the thiol moiety of one such cysteine is modified by oxidation or alkylation (cf. the Introduction section), hence the necessity to keep these residues in their reduced state, at least in the nuclear compartment. Several proteins have been identified that are able to reduce cysteine residues. For example, Trx, Ref-1 and glutaredoxin (thioltransferase) have been shown to increase the DNA binding of several transcription factors by targeting a crucial cysteine. Human Trx is a small multifunctional protein of 12 kDa [153]. It contains a conserved Cys-Gly-Pro-Cys sequence that confers redox activity by switching from dithiol to disulphide. It is thus able to reduce oxidized thiol groups located on a target transcription factor. Trx is therefore an antioxidant within the cell. When it is overexpressed, Trx protects the activity of several transcription factors from oxidative stress, as in the case of the GR and the ER, the iron-regulatory proteins and other important transcription factors described above. Conversely, antisense Trx sequences have a similar effect to oxidant reagents.

The 37 kDa Ref-1 protein is a multifunctional enzyme. It was identified as a nuclear protein that facilitates AP-1 DNA-binding activity [154]. This ubiquitous protein also appeared to have a DNA repair activity, and was named HAP-1 or APE (for apurinic/apyrimidic endonuclease). The latter activity is carried by the C-terminal part of the protein, whereas an N-terminal 61-amino-acid region is essential for the redox function of Ref-1. Cys-65 (in interaction with Cys-93) has been identified as the redox-active site [155]. It is notable that the analogous bacterial DNA repair enzymes lack the redox function. Similarly to Trx, Ref-1 can increase the DNA-binding activity of several transcription factors by reducing critical cysteines. Initially, the DNA binding of several transcription factors [Fos, Jun, cAMP responsive element binding protein (C/EBP), Myb and NF-$\kappa$B] was shown to be stimulated by Ref-1 [156]. The DNA-binding activity of several other transcription factors (Pax, nuclear factor-Y, HIF-1$\alpha$, etc.; see above) is altered by oxidative conditions and protected by Ref-1 [110,157,158]. In polyoma virus enhancer-binding protein 2/core binding factor (PEBP2/CBF), a transcription factor containing the conserved so-called Runt domain and involved in lymphoid cell differentiation, Cys-124 was identified as a critical redox sensor. Its oxidation inhibits the DNA-binding activity of the protein; Ref-1 prevents this oxidation [159].

The first reported case of a protective effect of Ref-1 on a protein was that of the AP-1 complex, which is composed of the Jun and Fos proteins. Although this transcription factor is activated by $H_2O_2$ [47,115], its DNA binding is altered if a critical cysteine (Cys-252 in Jun and Cys-154 in Fos) is oxidized. Several chemical reagents were shown to oxidize Cys-252 and alter AP-

1 DNA binding, e.g. diamide, *N*-ethylmaleimide [160] and NO [161]. Thioredoxin and Ref-1 help to maintain the integrity of this thiol group and can regulate the activity of AP-1. Indeed, in addition to its well-known activation of AP-1, phorbol ester treatment has been shown to induce translocation of Trx to the nucleus, where it interacts directly with Ref-1 [162]. The latter protein then interacts directly with Cys-252 of the Jun transcription factor. A similar mechanism could be envisaged for the activation of AP-1 by ROS. Interestingly, the mutation of this cysteine to a serine yields a Jun protein that is constitutively active, including under oxidative conditions. The redox modulation of wild-type Jun and the effects of Trx and Ref-1 contribute to the 'fine tuning' of AP-1 activity *in vivo*. Escaping from this control could enhance transforming activity [163]. Thus it appears that oxidative redox modulations control AP-1 in two distinct and opposite manners: stimulating transactivation and inhibiting DNA binding. Other transcription factors also display similar regulation. The transcription of the *egr-1* (early growth response 1) gene is induced by ROS [46], yet the protein contains three zinc fingers, and oxidized Egr-1 does not bind to its target DNA sequence *in vitro*. This oxidative repression is prevented by Ref-1 [164].

Finally, the transcription factor NF-$\kappa$B also displays complex redox regulation [165]. The cytosolic release of its inhibitor I$\kappa$B and subsequent NF-$\kappa$B nuclear translocation is induced by oxidative stress. In apparent contradiction to the activating effect of ROS, high levels of GSSG in T cells repress the DNA binding of NF-$\kappa$B [166]. Conversely, another study reported that the reducing proteins Trx and Ref-1 acted independently or synergistically to increase the *in vitro* DNA-binding activity of the p50 NF-$\kappa$B subunit. This regulation involved a critical cysteine residue (Cys-61) in the DBD of p50 [167,168]. In addition, co-transfection of a Trx-expressing plasmid was shown to increase the transcription of a reporter gene driven by the HIV long-terminal repeat sequence, in a NF-$\kappa$B-dependent manner [167]. However, it should be noted that another study using Trx overexpression in cultured cells showed that Trx could inhibit NF-$\kappa$B DNA binding [169]. The DNA binding of NF-$\kappa$B *in vivo* could thus be very sensitive to variations in the redox status within the cell, and needs fine redox tuning. Nonetheless, as with AP-1, it appears that NF-$\kappa$B may undergo opposite regulation by oxidative stress within the cytosolic and nuclear compartments. On the one hand these transcription factors are activated by ROS in intact cells, and on the other hand the oxidation of a critical cysteine may impair their DNA-binding activities, as observed *in vitro* [170]. It was shown in cultured cell lines that optimal activation of AP-1 and NF-$\kappa$B by ROS was obtained if there was a transient shift of the intracellular redox conditions towards a more oxidant status, followed by a rapid restoration of the redox homoeostatic conditions [170]. Dithiothreitol treatment 1 h after oxidative activation by ROS (or stimuli triggering ROS production) limited the inhibition of the DNA binding of these transcription factors. These apparent contradictory modes of regulation could be part of a feedback control loop that limits the activation of these ubiquitous and highly inducible transcription factors. Furthermore, it should be noted that the cytosolic and nuclear redox status can be different, owing to different GSH contents and to the function of redox proteins such as Trx and Ref-1. A cascade of redox interactions (thiol-disulphide exchanges) between these and possibly other, as yet unknown, redox proteins could influence gene transcription (Figure 5). Such a mechanism, and the translocation of redox proteins from the cytosol into the nucleus, could restore or maintain the activity of transcription factors under conditions of oxidative stress. Owing to the specificity of protein–protein interactions, fine control of the various redox-sensitive transcription factors is likely to be involved in the modulation of gene transcription by oxidative stress.

## CONCLUSIONS

### Differential redox regulation of transcription factors and gene expression

A growing number of transcription factors have been shown to be modulated by variations in the cellular redox status. However, the sensitivity of the different transcription factors to oxidation varies considerably, and this has seldom been taken into account. Indeed, the redox sensitivity of a cysteine residue is variable, according to its localization within the protein (exposed outside or hidden inside) and its amino acid environment. The latter may influence the sensitivity to an oxidant of the thiol moiety of the cysteine itself [171]. Because of the wide range of sensitivity of transcription factors, some of them are specifically down-regulated by an oxidative stress that is not cytotoxic. In this respect, ROS appear to be actual modulators of gene transcription, independently of the degradation of biological macromolecules. For example, when two transcription factors can bind to the same DNA promoter sequence, modulation of the intracellular redox status may differentially regulate their binding. Hence there is the possibility of a redox switch, allowing the replacement of a transcription factor by another one, according to their redox responsiveness. This is the case with the $\alpha$-globin gene proximal promoter, which is bound predominantly by NFI under normal conditions and by CP-1 under oxidative conditions. This redox switch is due to the particular sensitivity of NFI to the intracellular redox status. Other examples of competition between NFI and transcription factors that are activated or unaffected by oxidative stress have been reported ([93], and references therein). One is the switch between NF-$\kappa$B and Sp1, which have been reported to be able to compete for the same DNA sequences [172]. Such redox switches could provide an elegant mechanism for the regulation of gene transcription by ROS.

The TADs of transcription factors can be differentially regulated by $H_2O_2$. We observed that, at low (micromolar) concentrations that repress the TAD of NFI, those of Oct and Sp1 were unaffected (whereas higher concentrations have more general repressive effects). These differences in the modulation of transcription factor activities provide an explanation for the differential regulation of genes by oxidative stress.

The results reviewed above show that variations in the redox status within the cell can differentially regulate transcription factors. While positive sensors such as AP-1 or NF-$\kappa$B are activated by oxidative stress, other transcription factors, such as NFI, are repressed. These modulations may be useful in adaptation to a stress. For example, abnormally high $H_2O_2$ production within the cell causes a rapid increase in production of antioxidant enzymes (through AP-1, NF-$\kappa$B or the antioxidant-responsive element sequence binding proteins) and also the repression of ROS-producing systems (Figure 6). Several cases of such repression have been detailed above. The transcription of *CYP1A1* is repressed. Mitochondrial activity (and ROS release) is repressed because of the specific decrease in mitochondrial RNAs. The uptake of iron is limited by the decrease in transferrin receptor expression (owing to inhibition of IRP-1 RNA-binding activity). Another important regulation, occurring at the protein level, is repression of the activity of NADPH oxidase by oxidative modification of thiol moieties [173,174]. This repression is prevented by the antioxidant dithiothreitol. Thus it is likely that high ROS concentrations could have a repressive effect on the activity of this enzyme.

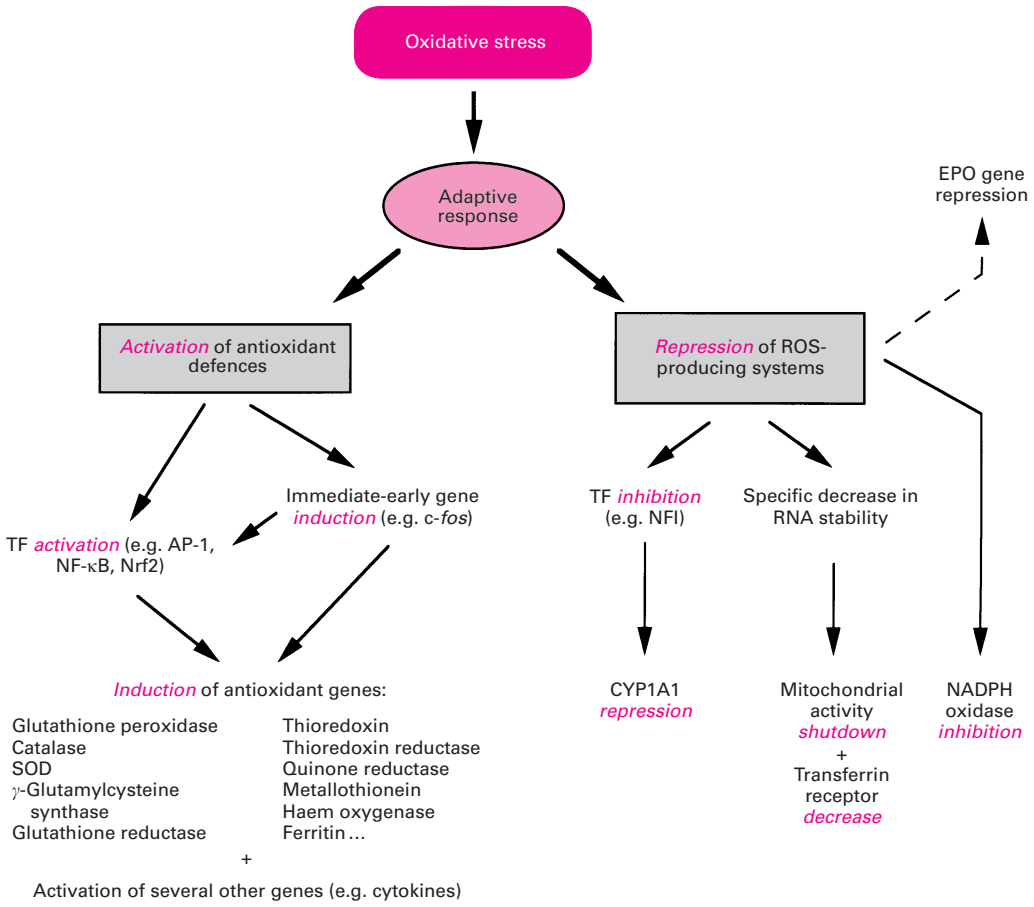


**Figure 6 Adaptive response to oxidative stress**

The cellular adaptive response to an oxidant insult comprises both the induction of antioxidant defences and the repression of endogenous ROS-generating systems or physiological pathways that indirectly increase the risk of ROS generation, such as the action of EPO, which increases oxygen uptake. TF, transcription factor; Nrf2, nuclear factor (NF)-E2 related factor 2.

The ROS-mediated regulatory process is rapid (it does not need any protein synthesis) and reversible (it involves simple chemistry). It also allows a common and transient response to several cellular stresses: radiation, inflammation, heat shock, xenobiotic influx. Indeed, the stimuli that can trigger intracellular ROS production have been shown to differentially regulate many genes. The differential display technique provides an interesting tool with which to assess the modulation of a large number of genes by a stimulus. Using this technique, UVB and serum (which contains several growth factors) were reported not only to activate immediate-early genes, but also to specifically repress others [61,175]. Such approaches could allow a global view of the influence of oxidative stress on gene transcription, in addition to the use of DNA array technology.

## Oxidative stress and disease

The chronic imbalance of the cellular redox status is associated with several pathological processes [176] and may alter physiological functions (as described above), such as the immune response, mitochondrial function, etc. We will focus here on some diseases involving gene repression by ROS. Endogenous ROS production rises with aging. Owing to altered enzymic reactions involving electron transport, electronic leakage is more important in cells from aged animals than in those from younger ones. Antioxidant systems are also less efficient (GSH and ascorbic acid levels in hepatocytes decline with age [5]). Therefore the redox status of the cell is chronically imbalanced, because it is shifted to a more oxidant state. Among other deleterious effects, this can chronically modify the function of transcription factors and cause a global alteration of gene expression. It has been shown that Sp1 activity was decreased in 30-month-old rats compared with that in young rats (whereas the activity of NF-$\kappa$B was increased) [134,177]. This may have important consequences, since Sp1 is a ubiquitous transcription factor often required for the expression of housekeeping genes. Other pathological conditions involving chronic inflammation are associated with an increase in ROS. The latter can alter immune functions by repressing several genes (see above). This is relevant in several diseases, such as arthritis [59] and HIV infection [178].

$H_2O_2$ has been shown to promote neoplastic transformation in a variety of tissues [44]. The production of ROS is a common feature of a wide range of tumour promoters, such as dioxin, UV, peroxisome proliferators, phorbol esters, okadaic acid, phenobarbital, etc. ([44], and references therein). In c-Myc/transforming growth factor $\alpha$ transgenic mice, marked ROS overproduction was shown to accelerate hepatocarcinogenesis [179]. In cancer cells, a chronic imbalance of the redox status, eliciting excessive ROS production, facilitates tumour promotion. Maximal growth promotion is observed when cells are protected from excessive toxicity but still maintain a sufficient oxidant signal for the induction of growth-competence genes [180]. Indeed, ROS have been described as inducers of proto-oncogenes such as c-*fos*, c-*jun* and c-*myc* ([44,181], and references therein).

However, the repressive effect of oxidative stress on gene expression is also implicated. As mentioned above, the oxidative inhibition of p53 function can, for example, prevent the activation of genes necessary to induce apoptosis or repair DNA alterations. It may thus facilitate the survival and further development of transformed cells. In addition, ROS interfere with the complex signalling mechanisms regulating mitosis and differentiation (cf. the above-mentioned inhibition of some cyclins). A deregulation of these processes may also increase cell transformation. Furthermore, long-term exposure to oxidative conditions leads to the accumulation of DNA damage, possibly leading to mutations. The guanine base is particularly sensitive to oxidation, and can be transformed into 8-oxoguanine; this is the case, for example, when CYP1A1 activity is high within the cell (and produces ROS) [97]. This could lead, after DNA replication, to a G → T transversion. The effect of a mutation within the coding sequence of a gene is well known: it can inhibit the function of the encoded protein. Less attention has been paid to mutations occurring within the regulatory sequences of the genes. However, the mutation or modification of a base in the 5′ upstream sequence of a gene can prevent the interaction of a transcription factor with its cognate DNA site, which may repress the transcription of the gene. Thus chronic oxidative stress can repress gene expression either by altering the activity of a transcription factor or by modifying a promoter sequence. It should also be noted that oxidative alterations of RNA nucleotides [182] may also be deleterious for protein expression, in addition to the effects of oxidation of RNA regulatory proteins.

### 'Redox-active' xenobiotics

Moderate but chronic oxidative stress can, as described above, result in modulation of gene expression, possibly leading to pathophysiological consequences. Various factors elicit oxidative stress. Apart from metabolic dysfunctions, environmental factors are critical. ROS production is increased upon intake of a high-calorie diet. Diet restriction (without malnutrition) was shown to decrease oxidative stress and increase the life span in animals [183]. Likewise, many xenobiotics (e.g. pollutants, food contaminants) cause ROS generation during their metabolism. Some compounds are strong inducers of enzymes (such as CYP family members) that may release ROS during their catalytic cycles. Thus the regular intake of antioxidants is currently thought to prevent, or at least limit, the deleterious effects of chronic oxidative stress (among which is altered gene expression). This has led to the recent development of drugs with antioxidant properties. These drugs may contribute to maintain a homoeostatic redox balance within the cell. In this respect, they may help to restore the correct regulation of gene transcription. Some results suggest that *N*-acetylcysteine, a precursor of glutathione, or direct cysteine complementation could help to buffer chronic oxidative stress (and its related deleterious effects), as is the case in cancer or in HIV-infected patients [184–186]. The antioxidant drugs mainly contain selenium (a necessary cofactor of glutathione peroxidase and Trx reductase), ascorbic acid (vitamin C), carotenoids (including vitamin A) and derivatives of α-tocopherol (vitamin E). A wide range of other antioxidant cocktails have been clinically tested, including various flavinoids. It is possible that the integration of such compounds in the diet decreases the risk of cancer (reviewed in [187]) or enhances immune function [188,189], but additional evidence is needed to support these conclusions.

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