



Oxidative stress signaling to chromatin in health and disease

Oxidative stress has a significant impact on the development and progression of common human pathologies, including cancer, diabetes, hypertension and neurodegenerative diseases. Increasing evidence suggests that oxidative stress globally influences chromatin structure, DNA methylation, enzymatic and non-enzymatic post-translational modifications of histones and DNA-binding proteins. The effects of oxidative stress on these chromatin alterations mediate a number of cellular changes, including modulation of gene expression, cell death, cell survival and mutagenesis, which are disease-driving mechanisms in human pathologies. Targeting oxidative stress-dependent pathways is thus a promising strategy for the prevention and treatment of these diseases. We summarize recent research developments connecting oxidative stress and chromatin regulation.

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Oxidative stress is involved in the pathogenesis of several diseases, like Alzheimer's and Parkinson's disease [1,2], amyotrophic lateral sclerosis [3], diabetes [4,5], hypertension [6,7], autoimmune disorders [8] or cancer [9,10]. Many of the pathogenic effects of oxidative stress are mediated by affecting chromatin.

Chromatin is a highly regulated signal integration platform, receiving inputs from extracellular and intracellular messengers and translating these into functional outputs such as activation or repression of transcription, DNA replication, chromatin condensation or DNA repair [11]. Among others, these processes are regulated by covalent modifications of chromatin, including DNA methylation (for a review see [12]) and modifications of histones, like methylation, acetylation, phosphorylation, ubiquitination, SUMOylation or poly-ADP-ribosylation (for a review see [13]). These epigenetic marks as well as specific DNA sequences or DNA

damage are recognized by effector proteins, which mediate the functional output.

Oxidative stress has been described to widely affect chromatin modifications as well as chromatin reader and repair proteins. These effects are thought to drive the physiological response to oxidative stress, but to also facilitate initiation and progression of several diseases. Here, we review how oxidative stress impacts on chromatin, how this is translated into a cellular outcome and how this is involved in common human diseases. Our analysis of the current data is focused on mechanisms directly linking oxidation or oxidative stress-mediated modification to chromatin and chromatin modifying enzymes. Effects of oxidative stress on upstream signal transduction pathways that indirectly influence chromatin have been omitted from our discussion.

Oxidative stress

In 2007, oxidative stress was redefined by Sies and Jones as 'an imbalance between oxidants

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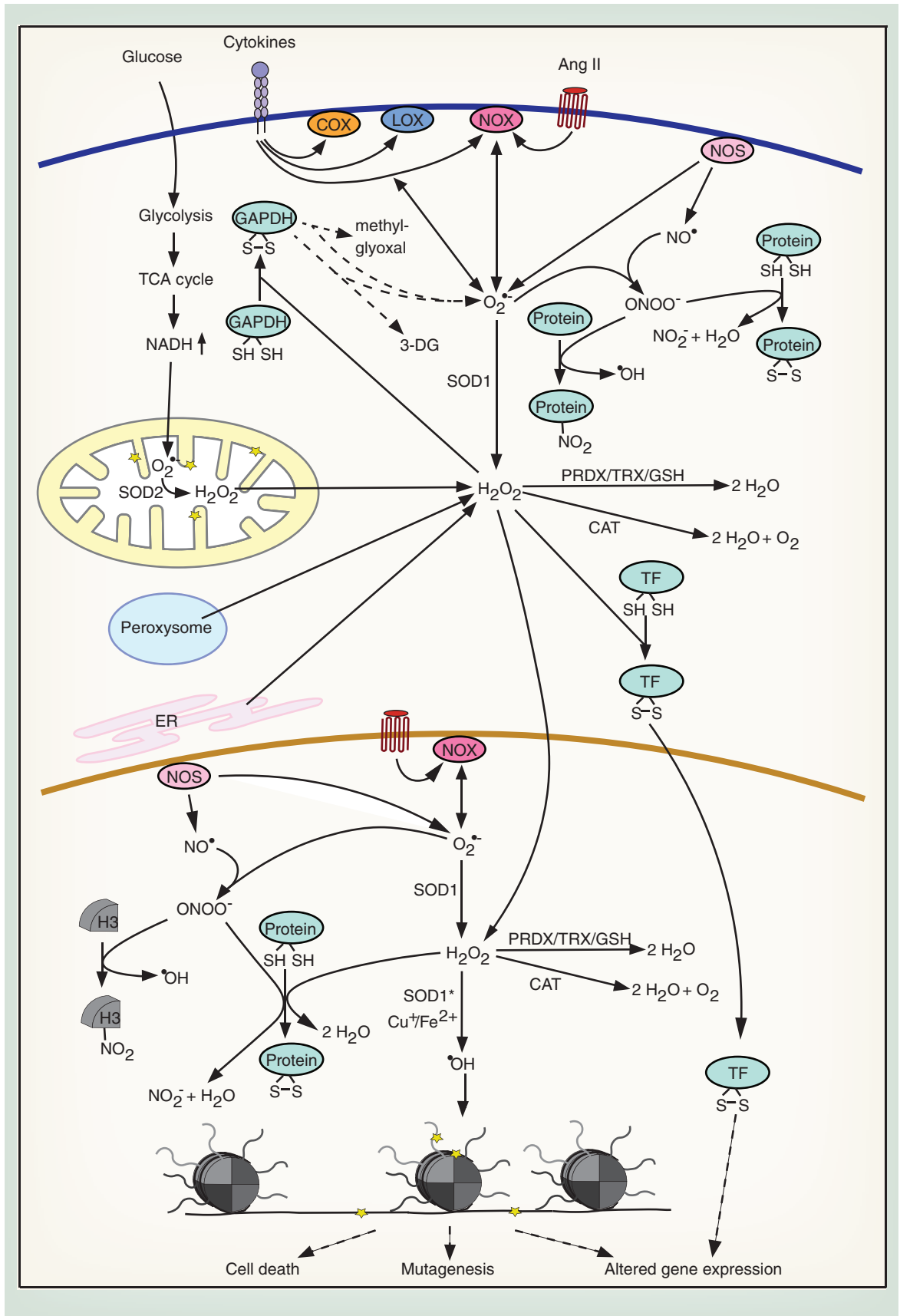


Figure 1. Causes and consequences of elevated nuclear and cytoplasmic reactive oxygen species (see facing page). Many pathogenic factors and pathophysiological processes lead to an increase in cytoplasmic and nuclear ROS. ROS can be detoxified, function as second messengers or damaging agents, altering gene expression, inducing mutagenesis or cell death. Stars indicate macromolecular damage.

3-DG: 3-deoxyglucosone; Ang II: Angiotensin II; CAT: Catalase; COX: Cyclooxygenase; ER: Endoplasmic reticulum; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GSH: Glutathione; LOX: Lipoxygenase; NO: Nitric oxide; NOS: Nitric oxide synthase; NOX: NADPH oxidase; PRDX: Peroxiredoxin; ROS: Reactive oxygen species; SOD: Superoxide dismutase; SOD*: Mutated SOD; TF: Transcription factor; TRX: Thioredoxin.

and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage' [14]. Physiologically, most cellular compartments are reducing environments [15,16], which are rich in antioxidants, like glutathione (GSH), ascorbic acid (vitamin C) or α -tocopherol (vitamin E) and antioxidant enzymes like peroxiredoxins, thioredoxins, superoxide dismutases (SODs) and catalase [17,18]. Consequently, most cysteines in intracellular proteins are in the reduced state under normal conditions to facilitate proper protein function [16,19]. Further, oxidative damage to DNA, RNA and lipids is prevented in such an environment because the antioxidant systems normally scavenge oxidants, like reactive oxygen or nitrogen species (ROS, RNS). ROS are produced when single electrons are transferred to oxygen, leading to the generation of the relatively inert superoxide radical ($O_2^{\cdot-}$) [20–23], the more reactive hydrogen peroxide (H_2O_2) and the highly reactive and damaging hydroxyl radical ($\cdot OH$) [24]. Damaging RNS result from the reaction of nitric oxide (NO^{\cdot}) with $O_2^{\cdot-}$, generating the highly reactive peroxynitrite ($ONOO^{\cdot}$) [25].

ROS & RNS as second messengers

Due to their intermediate reactivity, H_2O_2 and NO^{\cdot} are important physiological second messengers. Several signal transduction pathways activate NADPH oxidases (NOX) in the cytosol and the nucleus and their sole function is to produce $O_2^{\cdot-}$, which is then dismutated to H_2O_2 (Figure 1) [24,26,27]. H_2O_2 has limited toxicity because it selectively oxidizes cysteine residues in specialized protein environments, whose pK_a is particularly low, so that they exist as thiolate anions (S^-) at physiological pH [28,29]. Oxidized cysteines often form disulfide bonds, altering protein folding and function and thus serving as ROS sensors (Figure 1) [29]. This mechanism of redox regulation has been shown for a number of transcription factors, kinases, protein phosphatases and matrix metalloproteinases [30–33] (see [34,35] for reviews).

NO^{\cdot} is generated in the cytosol and the nucleus through the action of nitric oxide synthases (NOS) (Figure 1) [36,37]. NOS are dependent on Ca^{2+} /calmodulin and the cofactor tetrahydrobiopterin (BH_4). They catalyze the production of NO^{\cdot} from O_2 , arginine and NADPH [38]. Similar to H_2O_2 , NO^{\cdot} is not highly reactive toward macromolecules and is mainly implicated in physiological cellular signaling [39].

Pathways leading to increased ROS/RNS production

Although some ROS/RNS are important signaling molecules, overproduction of these molecules or pathological depletion of antioxidants can lead to oxidative stress. In eukaryotes, unintended ROS production is mainly a consequence of the oxidative metabolism. While oxygen itself has a very low reactivity [40], many cellular enzymes are capable of inadvertently transferring a single electron to O_2 , leading to generation of $O_2^{\cdot-}$ [20–23], which can damage biomolecules by either oxidizing or reducing them [24]. Physiologically, $O_2^{\cdot-}$ is rapidly detoxified by SOD in the mitochondria, the cytosol and the nucleus to yield H_2O_2 [20,41]. H_2O_2 is also produced by a number of metabolic enzymes, like the peroxisomal Acyl-CoA oxidase, or through protein oxidation in the ER (Figure 1) [42–44]. In contrast to $O_2^{\cdot-}$, H_2O_2 can diffuse through aquaporins to cross organelle membranes and enter the cytosol and the nucleus [45]. H_2O_2 is disproportionated by catalase or removed through the peroxiredoxin/thioredoxin and GSH systems (Figure 1) [46,47]. If H_2O_2 is not detoxified, it can be converted into $\cdot OH$ via the Cu^+ - or Fe^{2+} -catalyzed Fenton reaction. $\cdot OH$ is highly toxic and rapidly oxidizes and damages DNA, lipids or proteins (Figure 1) [29]. Similarly, $ONOO^{\cdot}$ damages proteins through tyrosine nitration and disruption of iron–sulfur clusters. Also, its potential to oxidize perceptible cytosines is much higher than that of H_2O_2 (Figure 1) [25].

One of the main causes of oxidative stress is mitochondrial dysfunction, resulting from damaged mitochondria [1,2,6,10] or from hyperglycemia-induced alterations in the cellular metabolism in diabetes (Figure 1) [4,5]. Other major contributors to oxidative stress are cytosolic and nuclear enzymes. Enhanced production of ROS by hyperactivated NOX, 'uncoupled' NOS or other enzymes, like lipoxygenase (LOX), cyclooxygenase (COX) or xanthine oxidase, can be the cause of oxidative stress [6,7,9]. Among others, the activation of NOX, COX and LOX can be driven by acute or chronic inflammation through the exposure of cells to cytokines [48,49] or by hormones like angiotensin II in hypertension (Figure 1) [50]. NOS can be uncoupled and release $O_2^{\cdot-}$ instead of NO^{\cdot} when cofactors are depleted or inhibited, when specific threonine residues are dephosphorylated or critical cysteines are oxidized [51–54].

Defects in the ROS detoxifying machinery can also significantly contribute to the generation of oxidative stress. In some cases of familial amyotrophic lateral sclerosis, mutations of SOD1 enhance its ability to dismutate H_2O_2 to $\cdot OH$ and to catalyze tyrosine nitration (Figure 1) [55,56]. Decreased expression of or mutations in catalase lead to oxidative stress in cancer and diabetes [57–59]. Apart from these enzymatic mechanisms for increased ROS production, non-enzymatic pathways are known. In Alzheimer's and Parkinson's disease intracellular accumulation of metals, like copper, iron and manganese leads to enhanced oxidative stress by catalyzing the Fenton reaction or by oxidizing dopamine, which results in ROS production [60]. Furthermore, exposure to toxic chemicals, such as cigarette smoke, can induce oxidative stress.

Importantly, there is extensive cross-talk between the different ROS-generating pathways, driving a vicious cycle of ever increasing cellular oxidative stress. Mitochondrial ROS can, for example, stimulate NOX-mediated ROS production via a PKC-dependent pathway and vice versa [61,62]. This interplay can potentially drive nuclear ROS production in the context of mitochondrial dysfunction. Moreover, increased mitochondrial ROS inhibit GAPDH through cysteine oxidation [63], causing an increase in all upstream glycolytic products. Under conditions of hyperglycemia (diabetes), this activates alternative arms of glucose metabolism, including the polyol and hexosamine pathways, which contribute to increased ROS formation (Figure 1) [4]. Also, COX is activated by ROS and is itself a source of ROS (Figure 1) [64].

Pathophysiological consequences of oxidative stress

An important pathogenic mechanism of oxidative stress in neurodegenerative diseases and diabetes is the induction of cell death [65–68]. ROS can participate in cancer development and progression by inducing DNA damage, which leads to mutagenesis, and by affecting the expression of oncogenes [69–71]. In diabetes, oxidative stress causes alterations in the cellular metabolism, contributing to the development of hyperglycemia-associated complications [4,5,63]. Moreover, ROS/RNS-protein and -DNA adducts can function as autoantigens in autoimmune disorders like systemic lupus erythematosus (SLE) [72,73]. In hypertension, reaction of ROS with $NO\cdot$ is a major pathogenic mechanism inhibiting vasodilation by $NO\cdot$ and generating the damaging $ONOO^-$ [74]. Major aspects of the ROS-dependent pathogenic mechanisms are mediated by affecting chromatin components.

Effects of oxidative stress on global chromatin structure

The global structure of chromatin is strictly organized within a cell. Based on different transcriptional activities, specific sets of chromatin modifications, associated proteins and distinct replication timings in S phase, at least four different chromatin states can be distinguished. These are transcriptionally active euchromatin and three distinct heterochromatic states: transcriptionally repressed chromatin, silent 'null' chromatin, which is not associated with specific proteins or histone marks, and HP1-associated heterochromatin [75]. Maintaining appropriate levels of the latter is important to facilitate genome stability [76,77], proper transcription [78,79], mitosis [80] and meiosis [81]. Oxidative stress can stimulate global heterochromatin loss and thus potentially affect these processes [82].

In this context, it has recently been shown that overexpression of tau, one of the pathogenic factors in Alzheimer's disease, causes oxidative stress-induced heterochromatin loss and neurotoxicity in a *Drosophila* model [83]. Mechanistically, it was hypothesized that oxidative stress-induced DNA damage promotes chromatin relaxation and thus a global reduction in heterochromatin in neurons. Heterochromatin loss then facilitates ectopic expression of normally silenced genes to induce cell cycle entry of postmitotic neurons [83], which is known to be neurotoxic [84]. In line with these findings, heterochromatin was reduced in neurons isolated from human post-mortem Alzheimer's brains and expression of heterochromatic, pluripotency-associated genes was increased [83].

Different mechanisms are in place to protect cells from heterochromatin loss and enhance genome stability upon exposure to oxidative stress. 293F cells, for example, show an increase in the stability of pericentromeric heterochromatin upon exposure to hydrogen peroxide. Pericentromeric heterochromatin is characterized by high levels of the histone H3K9 methyltransferase SUV39H1, the SIRT1 deacetylase and HP1 proteins. Hydrogen peroxide upregulates the expression of SIRT1, which increases SUV39H1 stability by inhibiting its ubiquitination and proteasomal degradation. Increased SUV39H1 stability is directly linked to its enhanced turnover at pericentromeric heterochromatin, which decreases DNA damage in response to stress [85]. This mechanism might play a role in the enhanced resistance of cancer cells to oxidative stress and it could possibly be exploited to inhibit heterochromatin loss in neuronal cells, ultimately preventing cell death.

Direct effects of oxidative stress on histones

Histones are extensively modified in an ROS- and RNS-dependent way and are glutathionylated in a

redox-sensitive manner, which affects their folding and stability, as well as their ability to be post-translationally modified. Because histones are the most common chromatin proteins, any change in their abundance, structure or post-translational modifications (PTMs) will have a severe impact on the global structure of chromatin, influencing gene expression, genome stability and replication.

Modification of histones by peroxynitrite

Histones can be directly modified by peroxynitrite. *In vitro* exposure of recombinant histones H1 and H3 to peroxynitrite leads to tyrosine nitration. Nitrated histones show an increase in structured domains, specifically β -sheet structures, and increased thermostability. This observed increased thermostability might contribute to protecting DNA from oxidative dam-

age during oxidative stress (Figure 2A) [86,87]. Further, nitrated H1 is a potent autoantigen in SLE and thus contributes to the pathogenesis of this disease [87].

Modification of histones by reactive aldehydes

Reactive aldehydes are generated intracellularly in an ROS-dependent way. They readily react with proteins to form carbonyl adducts. Lipids can be enzymatically or non-enzymatically peroxidized to yield highly reactive α,β -unsaturated aldehydes, such as glyoxal, malondialdehyde, acrolein, 4-hydroxy-2-nonenal (4-HNE) or 4-oxo-2-nonenal (4-ONE) [88]. Furthermore, ROS promote the production of the glucose metabolites methylglyoxal and 3-deoxyglucosone [89,90]. These reactive carbonyl species form adducts with permissive cysteines, arginines, lysines or histidines in proteins resulting in advanced glycation endproducts (AGEs)

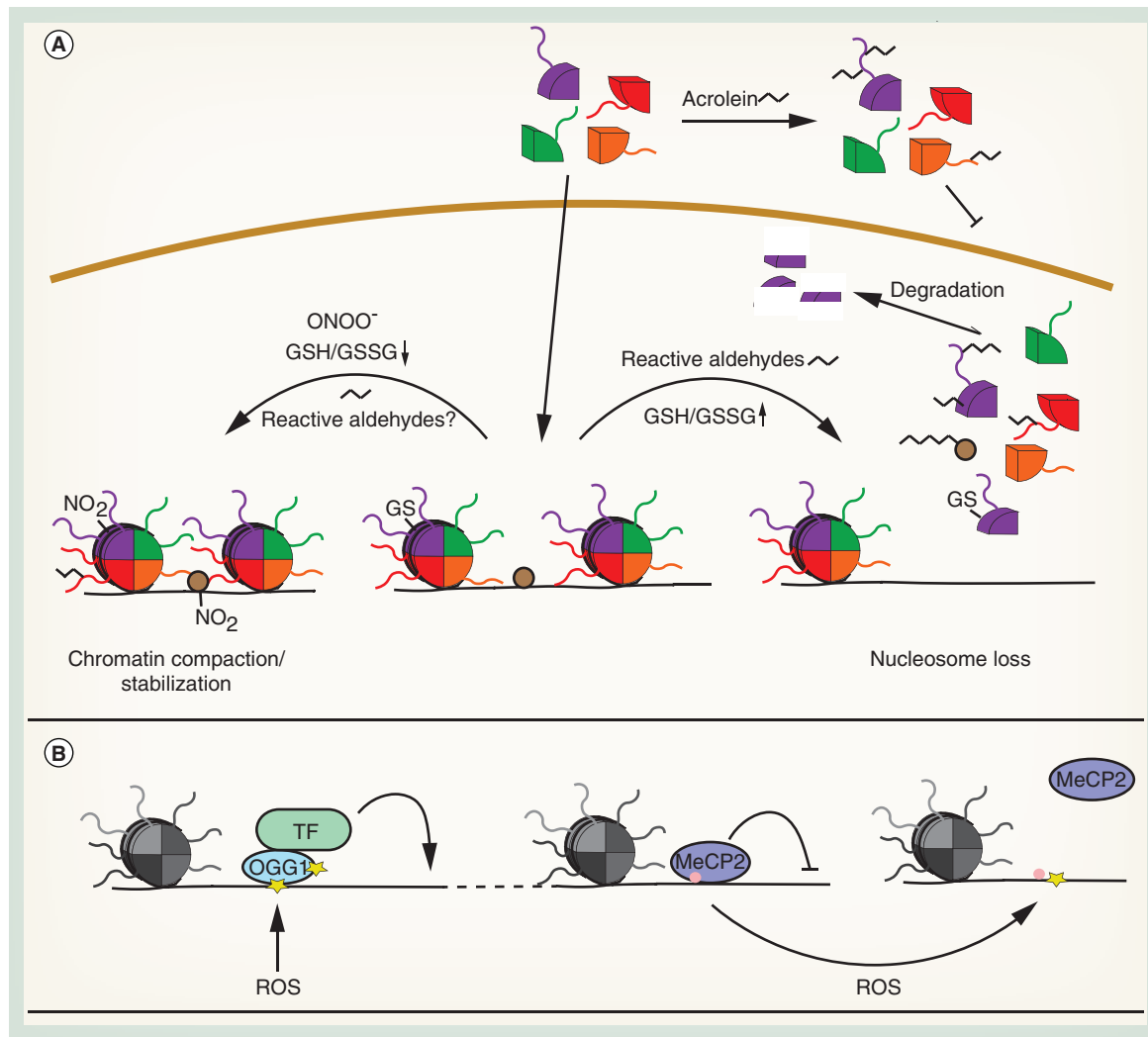


Figure 2. Oxidative stress induces direct modifications of chromatin. (A) Different oxidative histone modifications lead to alterations of chromatin structure. (B) Oxidation of deoxyguanine directly influences gene expression. GS: Reduced glutathione; GSH: Glutathione; GSSG: Glutathione disulfide; NO: Nitric oxide; ROS: Reactive oxygen species; TF: Transcription factor.

or advanced lipoxidation endproducts and facilitating protein cross-linking [91]. AGEs can further form under conditions of hyperglycemia through direct adduction of glucose or other sugars to proteins and ROS-dependent conversion to carboxymethyllysine or pentosidine [92,93]. Strong evidence indicates that carbonylated proteins are implicated in the initiation and progression of human pathologies. Accumulation of AGEs is, for example, associated with increased vascular stiffness in diabetic individuals, both in the macro- and microcirculation [94]. Increases in AGEs have also been described in Alzheimer's disease, where they might contribute to amyloid β crosslinking [95].

Histones are common AGEs/advanced lipoxidation endproducts and H2A modified by 4-HNE has been identified as an autoantigen in SLE [96]. *In vitro*, histones H1, H2A and H3 that have been modified with 3-deoxyglucosone become less thermostable due to partial unfolding. *In vivo*, this might lead to alterations in chromatin structure and gene expression [97–99]. In contrast, *in vitro* glycation of H2A by methylglyoxal led to an increase in alpha-helical structures and stabilization of the protein [100]. These discrepancies might be due to different target residues of the different reactive aldehydes or to the formation of different endproducts.

Several studies indicate that carbonylated histones are lost from chromatin, resulting in an overall reduction in nucleosome content. For example, H1 isolated from livers of diabetic rats displayed a reduced α -helical content and diminished binding to DNA [101], suggesting that the structural alterations described for H1, H2A and H3 *in vitro* can generally affect association of histones with DNA (Figure 2A). Similarly, increased cellular 4-ONE might trigger global nucleosome loss via inhibition of nucleosome assembly (Figure 2A). In contrast to 4-HNE, which targets a variety of cellular proteins, 4-ONE preferentially modifies histones. Among others, adducts are formed with H3K23, H3K27 and H4K79, inhibiting nucleosome assembly *in vitro*. The H3K27 adduct was also detected in LPS-stimulated macrophages, indicating that it might be a novel ROS-mediated, specific histone mark that stimulates transcription, rather than an unspecific, damaging adduct [102].

Another study in human bronchial epithelial cells described a different mechanism for global histone loss. Free histones are more sensitive to adduct formation with the cigarette smoke component acrolein than histones in nucleosomes. Because acrolein reacts with lysine and arginine residues, acetylation of newly synthesized histones was blocked, inhibiting their nuclear import and incorporation into chromatin (Figure 2A). Consistently, high concentrations of acrolein led to a

global reduction of chromatin-associated H3, which correlated with increased transcription at histone-depleted loci [103]. Moreover, glycated histones are irreversibly damaged and need to be removed by the nuclear proteasome [104], which possibly contributes to the global loss of histones from chromatin (Figure 2A).

Glutathionylation of histones

Glutathionylation is a common modification of proteins in oxidatively stressed cells. Cysteines oxidized to sulfenic acid can be glutathionylated by glutathione-S-transferases (GSTs), utilizing GSH, to protect the residues from further, irreversible oxidation to sulfonic acid (SO_3H) [105,106]. However, oxidative stress also increases the oxidation of GSH to GSSG, decreasing the GSH/GSSG ratio. Glutathionylation of histones H3 and H2B has been shown in MCF7 breast cancer cells and is most likely dependent on GSTP1–1. Enhanced glutathionylation correlated with higher GSH levels and drug resistance, suggesting that it might lead to altered expression of drug resistance genes [107].

In agreement with these findings, histone H3 is glutathionylated in proliferating cells, which is stimulated when the GSH/GSSG ratio is high, but decreases when GSH is depleted (Figure 2A). Glutathionylation of H3 decreases nucleosome stability, facilitates gene expression and DNA replication [108]. It is conceivable that an oxidative stress-induced reduction in glutathionylation may protect cells from oxidative stress by leading to chromatin compaction and inhibition of replication of potentially damaged DNA. Further, reduced histone glutathionylation might contribute to global gene regulation.

Oxidative damage to DNA

In addition to histones, DNA can also be directly affected by oxidative stress. The most susceptible nucleoside toward oxidation is deoxyguanosine (dG), which becomes easily oxidized to 8-oxo-dG. It is normally bound and excised by OGG1 and then repaired by the base excision repair pathway. Recent studies indicate that this oxidatively 'damaged' base can contribute to gene regulation upon oxidative stress.

G-quadruplex (G4) structures, which occur in certain G-rich DNA sequences and consist of guanine tetrads stabilized by hydrogen bonds and monovalent cations, are preferred targets for oxidation. Many oncogenes, like *MYC*, *KRAS*, *VEGF*, *BCL2*, *RB1* or *HIF1* are regulated by these G4 structures. Some transcription factors do preferentially bind to these sequences and alter the G4 conformational state to regulate gene expression [109]. Hypoxic stress, which leads to enhanced ROS formation, triggers oxidative damage

preferentially in G4-containing regions and about 25% of genes regulated by hypoxia contain G4 structures [110]. This might drive the preferential expression of the aforementioned oncogenes. In another study, a mechanism for 8-oxo-dG-induced transcription has been described. Treatment of airway epithelial cells with TNF α leads to enhanced ROS production and an increase in global 8-oxo-dG. OGG1 is also oxidized under these conditions, rendering it enzymatically inactive. Inactive OGG1 does, however, still bind 8-oxo-dG, recruiting transcription factors and enhancing gene expression (Figure 2B) [111].

Oxidative DNA damage can also inhibit binding of chromatin proteins. Methyl-CpG-binding proteins normally recognize methylated CpG dinucleotides and mediate transcriptional repression. However, binding of methyl-CpG-binding protein 2 to methylated CpG is inhibited when the guanosine in such a dinucleotide is oxidized or when methylcytosine is oxidatively converted to hydroxymethylcytosine (Figure 2B) [112].

Effects of oxidative stress on DNA methylation

In addition to directly modifying DNA, ROS can also affect DNA methylation, which is generally linked to repression of transcription. In mammals, the primary methylation targets are cytosines in CpG dinucleotides. While CpGs are usually methylated genome-wide, regions with a high density of CpG dinucleotides (CpG islands) are mostly hypomethylated [113]. Two classes of enzymes control DNA methylation: DNMTs and the demethylases of the TET family. DNMTs utilize S-adenosylmethionine (SAM) to transfer a methyl group to cytosine, while TETs oxidize 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) in an Fe(II)-, α -ketoglutarate- (α -KG), ascorbate- and O₂-dependent reaction [114,115].

In many pathologies, like Alzheimer's disease, diabetes or cancer global DNA hypomethylation has been described [116–119]. However, some specific CpG islands are hypermethylated in diabetes or cancer [117,120] and it has been suggested that both global hypomethylation and specific hypermethylation can be linked to oxidative stress.

DNA methylation is directly blocked when SAM decreases. SAM is synthesized from methionine by methionine adenosyltransferase (MAT). It is then used for methylation reactions, producing S-adenosylhomocysteine, which is hydrolyzed to homocysteine. Methionine is regenerated from homocysteine by methionine synthase (MS) (Figure 3). Depletion of SAM can be triggered by oxidative stress via several mechanisms. Firstly, oxidation of MAT leads to its inactivation and to reduced SAM synthesis from methionine [121,122].

Secondly, the cobalamine in MS is easily oxidized, which leads to inactivation of the enzyme. MS can be reactivated by MS reductase utilizing SAM, forming a futile cycle without any net SAM production [123]. And thirdly, the transsulfuration pathway can regenerate glutathione under oxidative stress conditions from SAM via homocysteine, cystathionine and cysteine, depleting SAM in the process (Figure 3) [124–126]. In agreement with this, SAM and DNA methylation were decreased in oxidatively stressed bladder cancer and kidney cell lines. There, supplying SAM, methionine or *N*-acetylcysteine restored DNA methylation [127]. Similarly, 5-mC levels in liver tissue of hamsters treated with the GSH-depleting agent bromobenzene were reduced. This hypomethylation was rescued, when the animals were supplied with methionine [128]. Both experiments indicate that a reduction in SAM, and not oxidation or modification of DNMTs, was the prime reason for reduced DNA methylation. However, direct oxidation of 5-mC to 5-hmC can also lead to DNA demethylation. 5-hmC inhibits maintenance methyltransferase DNMT1, thus blocking the proper inheritance of methylation patterns and leading to indirect demethylation of CpG sites [129].

A mechanism for DNA hypermethylation upon oxidative stress is the inhibition of TET proteins. As Fe(II)- and α -KG-dependent dioxygenases, they can be inactivated by iron oxidation. During the catalytic cycle, Fe(II) is oxidized to Fe(III) and Fe(IV) [115] and it was hypothesized that oxidized iron is regenerated by ascorbate [114]. Under oxidative stress conditions, this regeneration is perturbed, leading to inactivation of the enzyme and an increase in global 5-mC levels [130]. Consistent with this, 5-hmC is significantly reduced in oxidatively stressed SY5Y neuroblastoma cells [131]. Furthermore, *TET1* knockdown enhances cell death in murine cerebellar granule cells, indicating that TET1 inhibition by oxidative stress could be involved in the pathogenesis of neurodegenerative diseases [132]. Additionally, the Krebs cycle intermediates succinate and fumarate inhibit TETs competitively due to their similarity with α -KG [133]. This mechanism might play a role in hyperglycemia, which leads to an increase in fumarate due to impairments in the mitochondrial electron transport chain [134]. An increase in succinate can result from the inhibition of succinate dehydrogenase by oxidative stress (Figure 3) [135].

Both hypermethylation of CpG islands and global hypomethylation can also be mediated by relocalization of DNMTs. Base excision and mismatch repair proteins recognize 8-oxo-dG and recruit maintenance DNA methyltransferase DNMT1, which forms a complex with the SIRT1 deacetylase, the *de novo* DNA methyltransferase DNMT3B and the histone

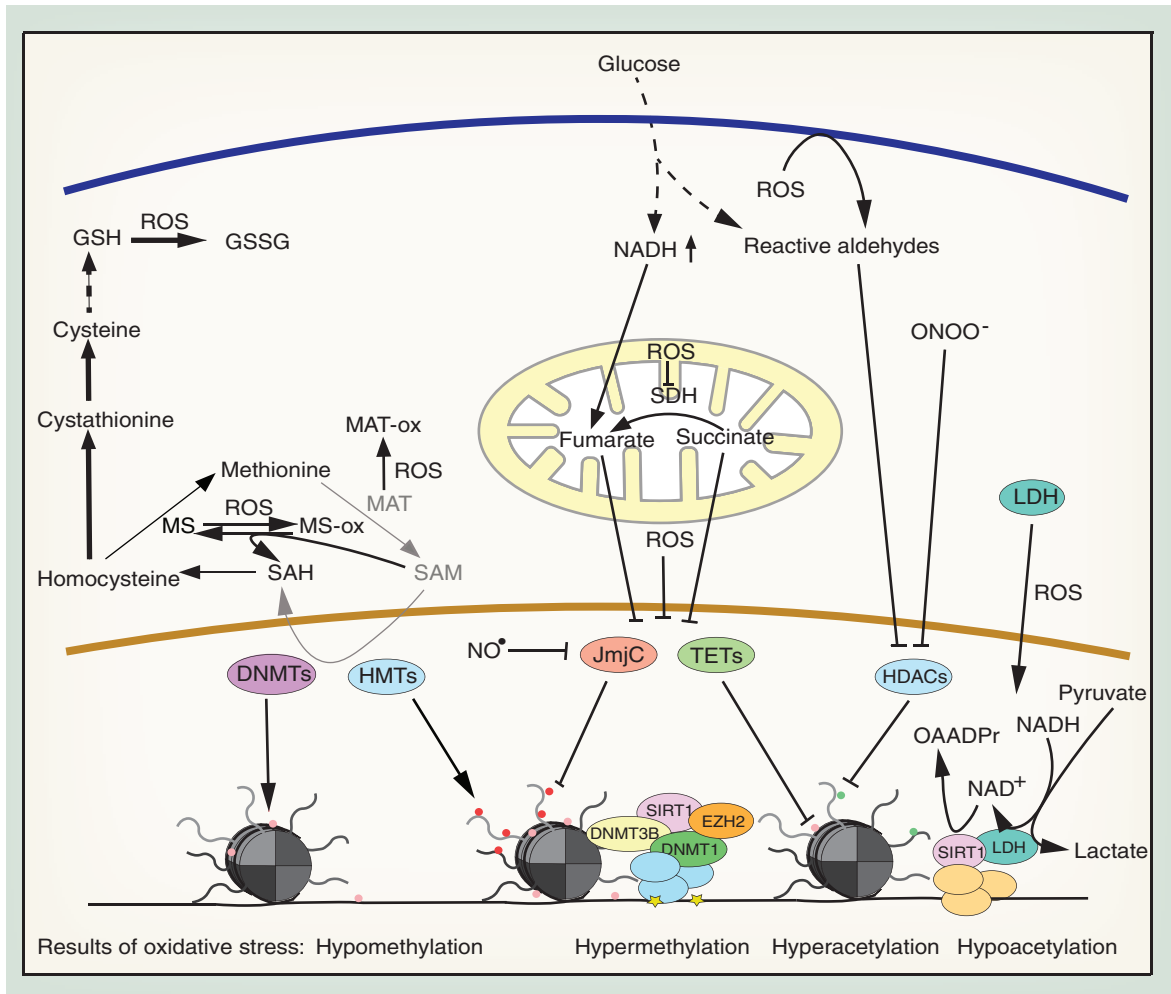


Figure 3. Oxidative stress influences chromatin modifiers. Chromatin modifying enzymes can be inhibited under oxidative stress conditions by different mechanisms, including SAM depletion, oxidation, carbonylation or nitration. Activation of DNA methylation is mediated by oxidative DNA damage and the SIRT1 HDAC can be stimulated by LDH.

DNMT: DNA methyltransferase; GSH: Glutathione; HDAC: Histone deacetylase; HMT: Histone methyltransferase; LDH: Lactate dehydrogenase; MAT: Methionine adenosyltransferase; MS: Methionine synthase; NAD⁺: Nicotinamide adenine dinucleotide; NO: Nitric oxide; ROS: Reactive oxygen species; SAM: S-adenosylmethionine; TET: Ten-Eleven-Translocation DNA demethylase.

H3K27 methyltransferase EZH2 [136,137]. The complex is mainly recruited to active CpG island promoter regions, where increased 8-oxo-dG is present, and it enhances DNA methylation and H3K27me₃, while reducing H3K4me₃ and H4K16ac levels (Figure 3). Simultaneously, DNMT1, DNMT3B and SIRT1 are lost from other genomic regions. As a consequence, CpG islands of lowly expressed genes were hypermethylated in an *in vivo* mouse colitis model after exposure to oxidative stress, while housekeeping genes remained unaffected. These same genes have also been shown to be hypermethylated in intestinal cancer, providing a mechanistic explanation for how oxidative stress can drive cancer-specific gene expression [136].

Effects of oxidative stress on histone modifications

Histone PTMs provide an additional layer of complexity for the regulation of chromatin structure and chromatin-regulated processes beyond the simple structural properties of histones. Covalent histone PTMs can directly or through the recognition by chromatin reader proteins influence transcription, chromatin structure, DNA repair or mitosis. Chromatin reader proteins are, for example, adaptor proteins or different enzymes, like chromatin modifiers or chromatin remodeling complexes [13]. Oxidative stress influences histone PTMs in various ways and thus affects a number of chromatin-regulated processes.

Histone lysine methylation

Lysines can be mono-, di- or trimethylated and histone lysine methylation is controlled by HMTs and HDMs. The latter can be divided into LSD and JmjC family enzymes. LSD enzymes catalyze demethylation of mono- and dimethylated lysines, JmjC proteins target all methylation states [138]. Especially JmjC family proteins can be inhibited by oxidative stress, leading to histone hypermethylation. The best-studied methylation marks are H3K4me1/2/3, H3K9me2/3 and H3K27me3. H3K4 methylation is generally associated with transcriptional activation, H3K9me2/3 are mainly found in constitutive heterochromatin and are associated with heterochromatin formation and maintenance, and H3K27me3 is associated with transcriptional silencing and facultative heterochromatin [13]. Alterations in these methylation marks can therefore result in aberrant transcription or changes in chromatin packaging.

Like DNA demethylases of the TET family, HDMs of the JmjC family are Fe(II)- and α -KG-dependent dioxygenases. As such, they are also inhibited by high levels of fumarate, which led to a global increase in H3K4me1, H3K4me3, H3K9me2/3 H3K27me2/3 and H3K79me2 in fumarate hydratase deficient cells [133,139]. Further, iron oxidation blocks the catalytic activity of JmjC HDMs (Figure 3) and especially H3K4me3 was significantly increased globally after exposure of human bronchial epithelial cells to hydrogen peroxide [130].

JmjC demethylases can also be inhibited by nitrosative stress. The NO[•] radical inhibits the JmjC domain-containing demethylase KDM3A by directly binding to the catalytic iron (Figure 3). Consequently, treatment of cells with NO[•] results in an increase in H3K9me2, which is normally targeted by KDM3A. Although NO[•] caused a simultaneous upregulation of KDM3A, this did not compensate for the NO[•]-dependent inhibition of the enzyme [140].

Lastly, HMTs could also be affected by oxidative stress. They are dependent on SAM and in oxidatively stressed cells reduced SAM levels are expected to block histone methylation and thus globally alter chromatin structure (Figure 3) [125,141].

It is important to note that oxidative stress does not influence all histone lysine marks in the same way. While some residues are hypermethylated in response to an oxidative insult, others are unaffected or even become hypomethylated [130]. This is intriguing because all known HMTs depend on SAM and all trimethylated residues are exclusively targeted by JmjC family HDMs. The observed specific effects might be a consequence of different enzymes having distinct sensitivities toward oxidation, nitration, competitive inhi-

bition or SAM depletion, depending on structural features of the enzyme or on the microenvironment of an enzyme complex. In this context, it has recently been shown that MATII can be recruited to target genes by the H3K9 methyltransferase SETDB1 [142]. This leads to a local increase in SAM and could facilitate histone methylation even in the absence of high global SAM levels. Hence, specific local rather than global regulation of HMT and HDM activities might predominate *in vivo*.

Histone acetylation

In contrast to methylation, acetylation of lysine residues neutralizes the positive charge of the histone tail and is therefore generally associated with chromatin relaxation and transcriptional activation. Global changes in histone acetylation patterns do thus have a broad and immediate impact on cell physiology and are intricately linked to several diseases. Histone acetylation is controlled by histone acetyltransferases and histone deacetylases (HDACs). HDACs are grouped into class I, II, III and IV enzymes, depending on their structure, homology to yeast HDACs, subcellular localization and catalytic mechanism [143–145]. Class I, II and VI enzymes possess a catalytic metal ion and produce free acetate [146], while class III HDACs or sirtuins require a structural zinc ion and NAD⁺, to which they transfer the acetyl group producing O-acetyl-ADP-ribose [147–149].

Oxidative and nitrosative stress are potent modulators of HDAC function at multiple levels. It has been shown that most class I HDACs (HDAC1, HDAC2 and HDAC3) are alkylated and inhibited by several reactive aldehydes, which results in changes in gene expression [150,151]. Moreover, HDAC2 can be nitrosylated [152], the effect of which is being discussed controversially. In one study conducted in neurons, nitrosylation did not influence its activity, but led to displacement from chromatin to activate gene expression [153]. In another study in C2C12 myoblasts, cysteine nitrosylation decreased HDAC2 activity [154]. In this context, it is interesting to note that knockdown or knockout of HDAC1, HDAC2 or HDAC3 can promote the development of hematological malignancies and hepatocellular carcinoma [155–158], indicating that ROS-mediated HDAC inhibition is potentially oncogenic. Furthermore, inhibition of HDACs by oxidative stress might be a physiological response to confer oxidative stress resistance to cells. Treatment of mice with the HDAC inhibitor β -hydroxybutyrate led to upregulation of FOXO3A and metallothioneine 2, two proteins that protect cells from oxidative stress [159].

Several class III HDACs are also inhibited by oxidative stress. SIRT1 can be inactivated as a result of

cysteine carbonylation [160], nitrosylation [152] or glutathionylation [161]. Inactivation of SIRT1 during oxidative stress leads to increased acetylation and inhibition of FOXO3. Activation or overexpression of SIRT1, in contrast, protects cells from oxidative stress-induced senescence [162,163], indicating that SIRT1 inhibition negatively affects oxidatively stressed cells. Inactivation of SIRT1 by cigarette smoke results in enhanced acetylation of NFκ-B and the increased expression of proinflammatory genes in macrophages [164]. Similarly, SIRT3 has been shown to be carbonylated and inactivated by 4-HNE [165]. Nuclear SIRT3 is rapidly degraded when cells are exposed to oxidative stress, leading to the upregulation of stress-induced genes [166]. Moreover, the tumor suppressor SIRT6 can be inactivated through peroxynitrite-mediated nitrosylation [167,168], which could be oncogenic.

A different mechanism of oxidative stress regulation has been described for class II HDACs. In cardiac myocytes HDAC4 and HDAC5 translocate from the nucleus to the cytoplasm in an ROS-dependent manner [169]. This process stimulates transcription of MEF2-dependent genes, which has been shown to trigger hypertrophic growth of cardiac myocytes [170].

Although ROS are potent inhibitors of HDACs they can also increase histone deacetylation by directly stimulating HDAC expression [171,172] or by indirectly enhancing HDAC activity. Treatment of HepG2 cells with H₂O₂ led to an increase in nuclear lactate dehydrogenase, which catalyzes the reduction of pyruvate to lactate using NADH and producing NAD⁺. Lactate dehydrogenase directly interacts with SIRT1 and provides the enzyme with NAD⁺ to stimulate deacetylation [173].

Histone phosphorylation

Phosphorylation of different histone serine, threonine and tyrosine residues plays a role in the regulation of gene expression, DNA repair and mitosis [174]. Several studies show that oxidative stress can indirectly influence global histone phosphorylation levels. Oxidative stress leads to the formation of DNA double-strand breaks, which induce the phosphorylation of H2AX to trigger DNA repair [175,176]. Additionally, one study showed that H₂O₂ treatment increases H2AX phosphorylation in an ATR-dependent way independent of the presence of double-strand breaks, suggesting that it fulfills a different signaling function in oxidatively stressed cells [177]. The effect of ROS on H3S10 phosphorylation is controversial. While oxidative stress increased H3S10p in monocytes and alveolar macrophages in a p38-, MSK1- and IKK2-dependent way [178], it inhibited H3S10 phosphorylation by VRK1 in SY5Y neuroblastoma cells through upregulation of

MKP2 [179]. The histone-targeting protein phosphatases PP1 and PP2A can be inhibited by oxidation of their catalytic metal ion [180]. Whether this is a mechanism by which oxidative stress can directly modulate histone phosphorylation levels needs, however, to be seen.

Regulation of chromatin-associated proteins

Regulation of TRIM28

TRIM28 or KAP1 is a global transcriptional co-repressor protein. It can be recruited to a number of transcription factors and interacts with HDACs and HMTs [181]. Two mechanisms of negative regulation of TRIM28 by oxidative stress have been described. The first one involves nuclear import of tyrosyl-tRNA synthetase in oxidatively stressed cells, where it sequesters TRIM28 and HDAC1 to facilitate transcriptional activation of DNA damage repair genes. This protects cells from oxidative stress-induced DNA damage (Figure 4A) [182].

The second mechanism involves oxidation HP1γ. HP1s bind H3K9me3 and have conventionally been described as essential proteins for heterochromatin formation and maintenance. They are homodimeric proteins, which interact with a number of other chromatin factors. HP1γ is associated with both eu- and heterochromatin. In euchromatin, it has been shown to regulate gene expression, both positively and negatively. It was recently demonstrated that HP1γ is oxidized upon induction of oxidative stress in cultured cells. Oxidation leads to reversible disulfide bond and covalent homodimer formation. This significantly enhances the interaction of HP1γ with TRIM28, sequestering it and inhibiting its repressive function toward a reporter gene (Figure 4A). Consistent with this, HP1γ is required to retain cell viability after an oxidative stress insult, suggesting that this mechanism also contributes to gene regulation upon oxidative stress [183]. Interestingly, TRIM28 heterozygous mice are obese and show signs of metabolic syndrome, like decreased glucose tolerance [184], indicating that a reduction in TRIM28 levels or availability, for example, by sequestration in oxidatively stressed cells, could be causative for this disease.

Regulation of HMGB1

HMGBs are structural DNA-binding proteins that can bend, loop or unwind DNA. They can mediate nucleosome remodeling, DNA accessibility and histone H1 displacement to facilitate transcriptional activation. HMGB1 interacts with H1 through its highly acidic C terminus [185]. The functions of HMGB1 can be regulated by reversible oxidation of the protein, which leads to disulfide bond formation and conformational

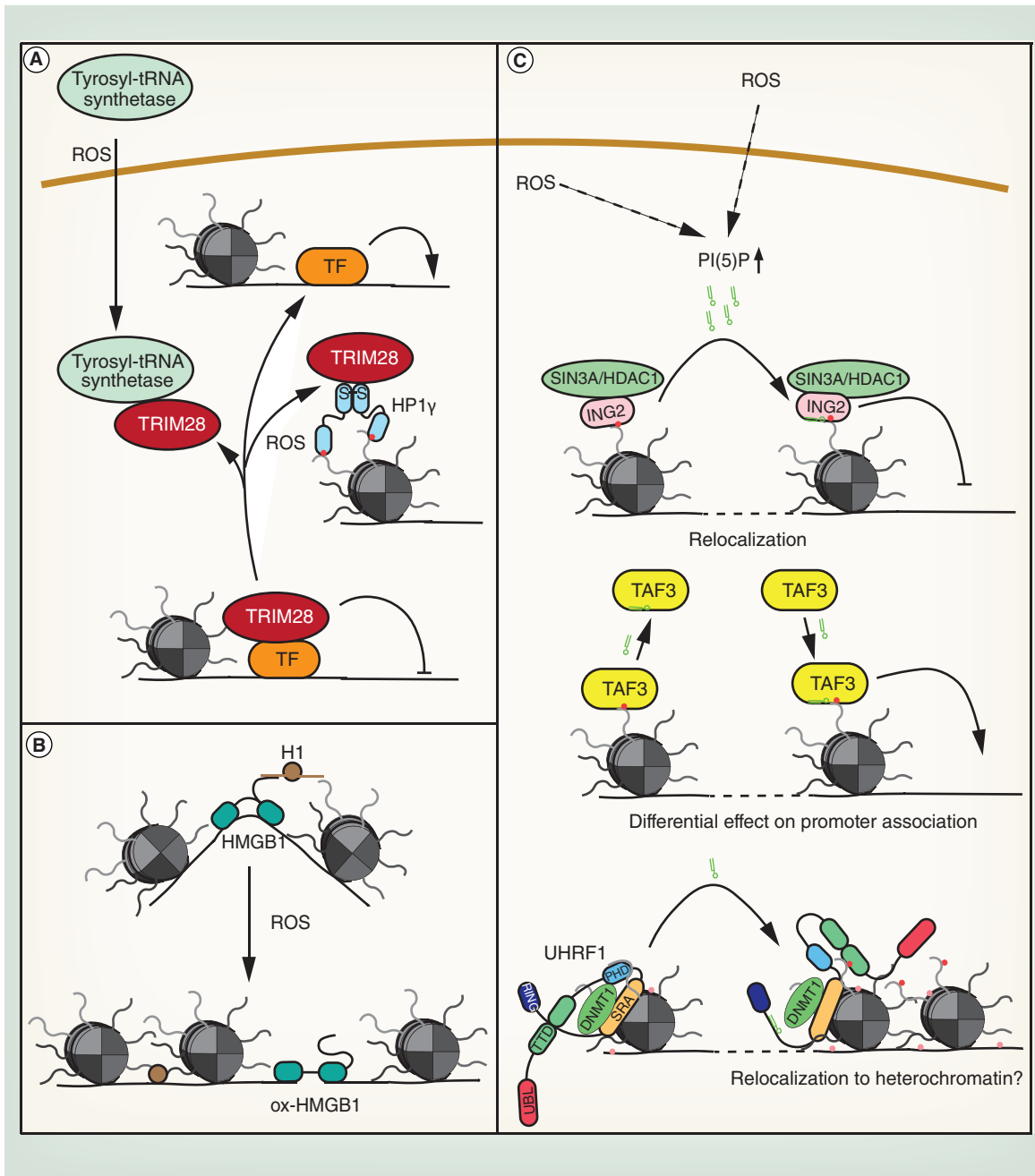


Figure 4. Effects of oxidative stress on different chromatin proteins. (A) Regulation of TRIM28 by oxidative stress. **(B)** Influence of oxidative stress on functions of HMGB1. **(C)** Regulation of chromatin proteins by PI(5)P. PI(5)P: Phosphatidylinositol-5-phosphate; ROS: Reactive oxygen species; TF: Transcription factor.

changes in the DNA-binding and -bending domain. Consistent with this, oxidized HMGB1 shows a two-fold lower DNA-binding affinity and loses its ability to bend DNA. It does, however, gain a DNA end-joining function, potentially increasing DNA repair after an oxidative insult. It also shows reduced affinity toward histone H1 and its ability to displace H1 from DNA is significantly inhibited (Figure 4B). In response to oxidative stress, stabilization of H1 on chromatin

might repress transcription and enhance chromatin stability [186]. Further, reduced bending of DNA by HMGB1 might result in transcriptional repression through impaired interactions of regulatory elements.

Regulation of chromatin-binding proteins by the second messenger PI(5)P during oxidative stress Phosphatidylinositol phosphates are important second messengers in a number of signal transduction

pathways. They are present in all cellular membrane compartments and, surprisingly, also in the nuclear matrix. Enzymes that produce mono-, di- and tri-phosphorylated PI have been detected in the nucleus, suggesting that the nuclear pool can be regulated independently of the membrane pool [187,188]. Indeed, recent publications highlight the specific functions of nuclear phosphatidylinositol phosphates as signaling molecules. PI(5)P increases in response to H₂O₂ and etoposide treatment [189], which mediates its effects by inducing oxidative stress and oxidative stress-dependent DNA damage [190]. PI(5)P might be a general mediator of the oxidative stress response, targeting a number of different chromatin proteins.

PI(5)P binds to ING2 [191], which specifically recognizes H3K4me3 and can associate with the p300 histone acetyltransferases [192] or the SIN3A–HDAC1 complex [193] to stimulate or repress transcription. PI(5)P binding to the ING2 plant homeodomain (PHD) triggers its relocalization to novel chromatin sites and is essential for repression of proproliferative genes [194] and the induction of apoptosis upon etoposide treatment (Figure 4C) [191]. Alterations in the transcriptional program can also be mediated by PI(5)P-dependent regulation of the general transcription factor TAF3 by either increasing or decreasing binding to promoter elements (Figure 4C) [195].

Another chromatin protein that interacts with PI(5)P is UHRF1. UHRF1 interacts with DNMT1, it is implicated in maintenance DNA methylation and stimulates proliferation in breast, prostate and lung cancer [196]. UHRF1 is composed of five domains, an N-terminal ubiquitin-like domain, an H3K9me3-binding tandem tudor domain (TTD), a PHD, which binds the unmodified N-terminal histone H3 tail, a hemi-methyl-CpG-binding SRA domain and a C-terminal RING domain. These domains are connected by flexible linkers. PI(5)P binding to the UHRF1 polybasic region in the linker between the SRA and RING domains alters the overall conformation of the protein. While the TTD is blocked in the non-PI(5)P bound protein, enabling binding of the unmodified H3 N-terminus to the PHD only, PI(5)P unblocks the TTD domain, allowing for binding of the TTD to K9me3-modified histone tails (Figure 4C) [197]. This mechanism might protect the genome from oxidative insults by facilitating heterochromatin and DNA methylation maintenance under conditions of oxidative stress. Furthermore, relocalization of UHRF1 to H3K9me3-marked chromatin might alter gene expression programs in stressed cells.

Conclusion & future perspective

As we have discussed, oxidative stress globally influences chromatin on multiple levels, from DNA and

histones to histone modifiers and structural DNA-binding proteins. Also, it is well known that oxidative stress is involved in initiation and progression of neurodegenerative diseases, diabetes, hypertension, cancer and several other pathologies. Nevertheless, for most mechanisms of chromatin regulation by oxidative stress highlighted in this review, a direct link to diseases has not been demonstrated. By and large, general effects of ROS have been described in different tissue culture model systems. Since the chromatin changes induced by oxidative or nitrosative stress observed there are also seen in human diseases, it can be projected that ROS-mediated chromatin alterations play an important role in initiation and progression of the aforementioned pathologies. Future research needs to focus on directly demonstrating how oxidative stress-induced chromatin changes impact on human diseases. Important questions to address are: how do ROS and RNS affect chromatin in specific cells in a disease setting? What are the molecular mechanisms for the observed oxidative stress-induced chromatin changes? What are the cellular effects of these chromatin changes? And what is the pathogenic potential of these chromatin-mediated effects?

Once these issues are resolved we are confident that it will be possible to exploit oxidative stress-mediated chromatin changes in diseases to develop drugs targeting specific ROS-induced chromatin signaling pathways. Antioxidants have already been widely tested as a treatment for neurodegenerative diseases, diabetes and cancer, but results were contradictory, ranging from improvement of the disease to accelerated progression [198–202]. Consistent with this, ROS have both protective and damaging effects on cells. To be effective, it will indeed be important to only target distinct parts of the ROS-dependent response. In this context, chromatin is a very interesting target for drug development because it integrates and mediates most cellular responses to extra- and intracellular stimuli. Furthermore, a number of small molecule inhibitors of chromatin modifying enzymes are already in clinical trials or are even being used as successful therapeutics, making them readily available for therapy. We are looking forward to the many basic connections between ROS, chromatin and human pathologies to be made and to their translation into medical applications.

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Executive summary

Oxidative stress

- Oxidative stress is an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage. It is linked to the initiation and progression of several common human diseases.

Oxidative stress & global chromatin structure

- Depending on the cell type, oxidative stress triggers global heterochromatin loss and cell death or protective stabilization of heterochromatin.
- Direct oxidative modifications of histones alter the global chromatin structure, induce changes in transcription, DNA replication and might regulate cell death.

Oxidative stress & DNA methylation

- Oxidative stress-induced inhibition of DNA methyltransferases and relocalization of DNA methyltransferases to CpG islands induces global DNA hypomethylation and local promoter hypermethylation.
- Inhibition of Fe(II)- and α -KG-dependent TET DNA demethylases by oxidation, fumarate or succinate leads to altered DNA methylation.

Oxidative stress & histone modifications

- Oxidative stress inhibits Fe(II)- and α -KG-dependent histone demethylases of the JmjC family and potentially triggers histone hypermethylation.
- Oxidative stress directly inhibits histone deacetylases, but also stimulates their expression and locally increases NAD⁺ to activate SIRT deacetylases.

Oxidative stress regulates DNA-associated proteins

- The corepressor TRIM28 is sequestered from the chromatin under conditions of oxidative stress by binding to tyrosyl-tRNA synthetase or oxidized HP1 γ , leading to activation of gene expression.
- Oxidized HMGB1 loses its DNA-bending activity and its ability to displace histone H1, possibly inhibiting gene expression and stabilizing chromatin. At the same time, it gains a DNA end-joining function, likely contributing to DNA repair after an oxidative insult.
- The second messenger PI(5)P is upregulated by oxidative stress and modulates the functions of the corepressor ING2, the general transcription factor TAF3 and the structural chromatin protein UHRF1, thus controlling transcription at multiple levels.

Future perspective

- Oxidative stress widely affects chromatin and is implicated in several human diseases. Further studies are required to comprehend the exact molecular connections of these phenomena for exploiting the management of oxidative stress-induced alterations for therapeutic purposes.

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