Article:
El-Khamisy, S.F. (2023) Oxidative DNA damage and repair at non-coding regulatory regions. Trends in Cell Biology, 33 (11). pp. 939-949. ISSN: 0962-8924

https://doi.org/10.1016/j.tcb.2023.03.004

Trends in
Cell Biology

CellPress

Review

Oxidative DNA damage and repair at non-coding regulatory regions

Sherif F. El-Khamisy [1,2,*]

DNA breaks at protein-coding sequences are well-established threats to tissue homeostasis and maintenance. They arise from the exposure to intracellular and environmental genotoxins, causing damage in one or two strands of the DNA. DNA breaks have been also reported in non-coding regulatory regions such as enhancers and promoters. They arise from essential cellular processes required for gene transcription, cell identity and function. One such process that has attracted recent attention is the oxidative demethylation of DNA and histones, which generates abasic sites and DNA single-strand breaks. Here, we discuss how oxidative DNA breaks at non-coding regulatory regions are generated and the recently reported role of NuMA (nuclear mitotic apparatus) protein in promoting transcription and repair at these regions.

Highlights

Endogenous oxidative DNA breaks arise at non-coding regulatory DNA regions such as enhancers and promoters.

The oxidative demethylation of DNA and histones is a potential source of DNA breaks at non-coding regulatory regions.

Nuclear mitotic apparatus (NuMA, also known as NUMA1) promotes transcriptional responses and repair of oxidative breaks at enhancers and promoters.

The failure in repair disrupts gene transcription and interferes with normal physiology.

DNA damage at non-coding gene regulatory regions

Epigenetic reprogramming is required to switch genes on and off during development and in response to stress. This involves modifications to the DNA itself and to histones. The oxidative demethylation of DNA and histones is emerging as a key source of endogenous DNA strand breaks at non-coding regions. Oxidative demethylation generates reactive oxygen species (ROS) that could damage proximal DNA sequences and produces DNA repair intermediates that if not repaired, cause DNA single-strand breaks. Transcription also requires overcoming topological constraints by DNA topoisomerases. The presence of unrepaired oxidative breaks prevents topoisomerases from completing their catalytic cycle, resulting in DNA strand breaks. The review discusses recent evidence showing how oxidative demethylation and topoisomerase 1 cause DNA single-strand breaks and the role of the nuclear mitotic apparatus (NuMA) in promoting transcription and repair responses.

DNA demethylation

The most prominent DNA modification is the methylation of carbon-5 of cytosine (5-methyl cytosine; 5mC), which is present in about 80% of CpG sites in the mammalian genome [1]. The steady-state level of 5mC regulates transcription and is controlled by DNA methylation via DNA methyl transferases, primarily DNMT1 and DNMT3 [2]. The level of 5mC could be 'passively' diluted during DNA replication or 'actively' removed by ten-eleven translocation (TET) proteins [3]. TET proteins undertake oxidative demethylation using molecular oxygen to successively oxidise 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Thymine DNA glycosylase (TDG) catalyses the excision of 5fC and 5caC to abasic sites (AP-sites) [4,5].

In addition to AP-sites produced as intermediates of cytosine demethylation, the demethylation reaction *per se* involves the formation of a reactive intermediate complex, Fe(IV)-oxo, which is an iron derivative of the hydroxyl radical (•OH) and superoxide anion radicals ($\bullet O_2$–) that can oxidise DNA bases [6,7]. The guanine base of DNA is the most vulnerable to ROS, producing 8-oxoguanine (8-oxo-G), which is the predominant DNA oxidative lesion in the genome [8]. If

[1]School of Biosciences, The Healthy Lifespan and Neuroscience Institutes, Firth Court, University of Sheffield, Sheffield, UK
[2]Institute of Cancer Therapeutics, School of Pharmacy and Medical Sciences, University of Bradford, Bradford, UK

*Correspondence:
s.el-khamisy@sheffield.ac.uk
(S.F. El-Khamisy).

not repaired, 8-oxo-G could lead to mutations (e.g., G>T), which can be deleterious and thus actively cleaved by DNA glycosylases, primarily 8-oxoG DNA glycosylase 1 (OGG1), also resulting in AP-sites [9].

AP-sites can block the progression of RNA polymerases during transcription and can be bypassed by trans-lesion DNA polymerases during replication, but in an error-prone manner [10,11]. Thus, the removal and subsequent repair of AP-sites are critical for the maintenance of genomic integrity. AP-sites are cleaved by AP endonuclease I (APE1) and tyrosyl DNA phosphodiesterase 1 (TDP1) [12]. If not enzymatically cleaved, AP-sites are unstable and can undergo spontaneous β-elimination to yield a single-strand break bearing a 3′-phospho-α,β-unsaturated aldehyde, which can also be removed by TDP1 and APE1 [13]. The single-strand break repair scaffold, X-ray repair cross-complementing protein 1 (XRCC1) and the activity of poly-ADP-ribose polymerase (PARP) are required during the removal and repair of AP-sites [14,15]. The requirement for PARP activity is most likely to relax nucleosome compaction and help concentrate repair factors at sites of damage (Figure 1A) [16].

Contrary to the assumption that AP-sites would accumulate at enhancers and promoters due to transcriptional activation, genome-wide mapping showed that these regulatory regions are protected, suggesting the presence of an active repair process [17,18]. We recently reported a mechanism that safeguards promoters and enhancers from oxidative DNA damage, which is mediated by NuMA (also known as NUMA1). NuMA is enriched at enhancers and approximately 100 bp either side of transcription start sites. It binds TDP1, XRCC1 and PARP1, and the binding is increased on chromatin following oxidative damage. TDP1 enrichment at damaged chromatin is facilitated by NuMA. Loss of NuMA in human retinal pigment epithelial-1 (RPE-1) cells and induced pluripotent stem cell (iPSC)-derived neurons increased oxidative damage and AP-sites at enhancers and promoters [18].

The involvement of cytosine demethylation as a source of breaks at non-coding regulatory regions is supported by observations that TET1 binds APE1 and PARP1 on chromatin [7]. Furthermore, by measuring the non-replicative incorporation of the nucleotide analogue 5-ethynyl-2′-deoxyuridine (EdU) as a proxy for DNA repair in iPSC-derived neurons, thousands of DNA repair hotspots were identified at enhancers and promoters [19,20]. The repair hotspots overlapped with sites identified by ATAC-seq, which marks regions of accessible chromatin. Reid *et al.* [19] found a strong enrichment of repair sites at promoters and at regions with high levels of H3K27 acetylation, a histone mark associated with active promoters and enhancers. However, Wu *et al.* [20] only observed strong overlap of repair peaks with sites of neuron-specific enhancers. The repair peaks coincided with poly-ADP-ribose ChIP-seq peaks and with peaks of XRCC1, suggesting an active repair process.

The repair peaks observed by Wu *et al.* [20] coincided with CG-rich regions, 5hmC and 5caC, pointing at cytosine demethylation as the source of the repair peaks. This repair event may be particularly relevant to long-lived neurons because they possess a high load of 5hmC [21]. It suggests that the optimal balance of cytosine methylation is maintained by methylation and demethylation, and because of the latter AP-sites are produced which requires timely repair by NuMA and the single-strand break repair machinery. Consistent with this idea, an elegant recent report [22] has shown that depletion of TDG in iPSC-derived neurons decreased, but did not completely abolish, the DNA repair peaks.

Histone demethylation

Transcriptional activation requires demethylation and acetylation of histones H3 and H4. This is particularly important for enhancer activity, which is marked by the removal of H3K27me3 and

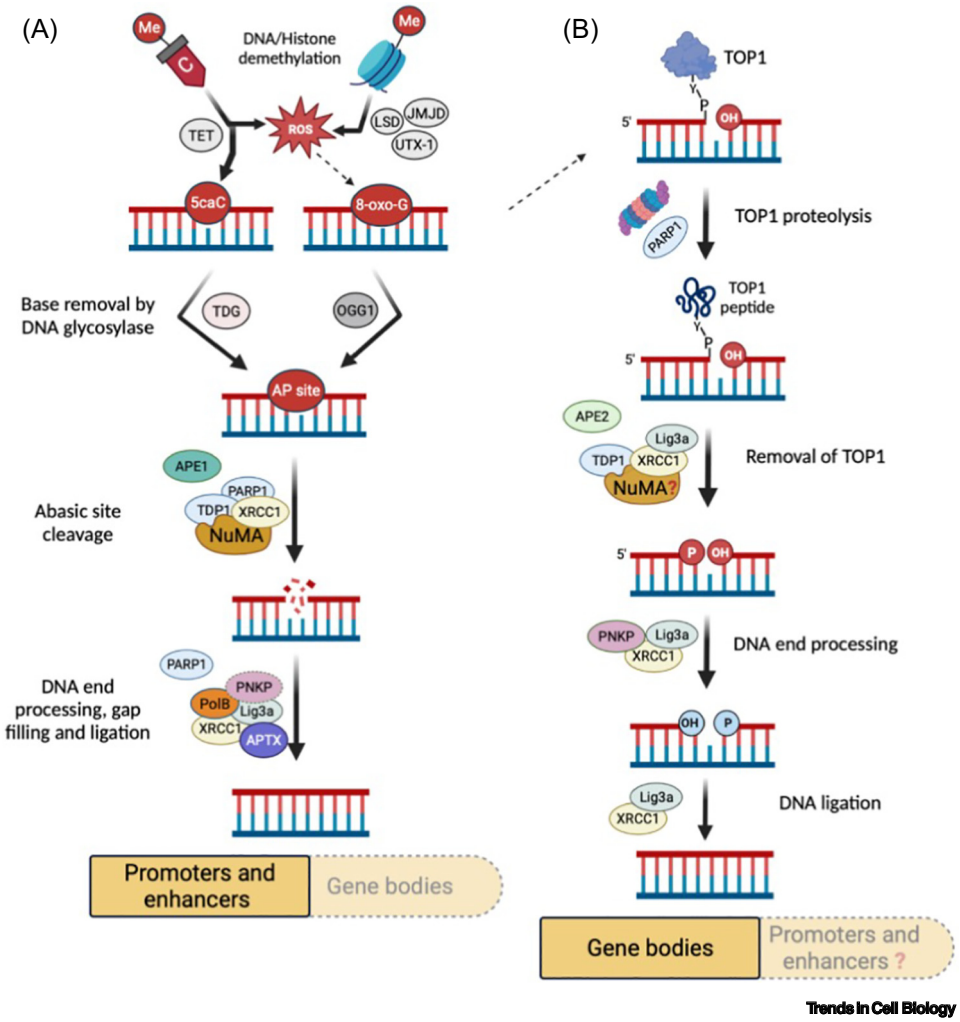

Figure 1. Sources and repair of oxidative and topoisomerase 1 (TOP1) DNA single-strand breaks. (A) Oxidative DNA demethylation, primarily of 5-methyl cytosine (shown as 'C'), by the ten-eleven translocation (TET) proteins generates 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine (5caC). Thymine DNA glycosylase (TDG) catalyses the excision of 5fC and 5caC to abasic sites (AP-sites). The DNA demethylation reaction also produces reactive oxygen species (ROS) as by-products, which damages the DNA, predominantly resulting in 8-oxoguanine (8-oxo-G). 8-oxoG DNA glycosylase 1 (OGG1) cleaves 8-oxo-G, also resulting in AP-sites. The demethylation of histones by lysine-specific demethylase (LSD) and Jumonji demethylases (JMJD, UTX-1) generates ROS and 8-oxo-G, resulting in AP-sites. AP-sites are processed by AP endonuclease I (APE1) and tyrosyl DNA phosphodiesterase 1 (TDP1) in a process that is facilitated by nuclear mitotic apparatus (NuMA), poly-ADP-ribose polymerase 1 (PARP1) and X-ray repair cross-complementing protein 1 (XRCC1). Subsequent end processing, gap filling and ligation take place by the action of polynucleotide kinase-phosphatase (PNKP), aprataxin (APTX), DNA polymerase B (PolB) and DNA ligase IIIa (Lig3a). The role of NuMA at non-coding regulatory regions is most pronounced at a subset of genes that need to respond promptly to stress, such as immediate early response genes. (B) Transcription activation and reprogramming requires the removal of topological stress, which is carried out by DNA topoisomerases [TOP1 and topoisomerase 2 (TOP2)]. DNA strand breaks are formed as part of the topoisomerase catalytic cycle, which are normally reversible, but on several occasions (e.g., proximity to AP-sites or 8-oxo-G) become irreversible and require enzymatic intervention (TOP1 is shown as an example). The repair is initiated by proteolytic degradation of TOP1 to a small peptide, followed by removal of the TOP1 peptide by TDP1, then DNA end processing and ligation. Abbreviations: APE2, AP endonuclease II; UTX-1, ubiquitously transcribed tetratricopeptide repeat, X chromosome.

deposition of activating H3K27ac mark to maintain an open chromatin state at enhancers and promote enhancer RNA synthesis [23]. Histone demethylases belong to two classes. flavin adenine dinucleotide-dependent monoamine oxidases, which includes lysine-specific demethylase

1 (LSD1, also known as KDM1A). LSD1 demethylates H3K4me and H3K9me at target loci in a context-dependent manner and generates H_2O_2 as a by-product [24]. The second class is the Jumonji (JMJ) family, which also generates ROS as a by-product in the form of superoxide anion ($\bullet O_2^-$) during oxidative decarboxylation of α-ketoglutarate, which depends on molecular oxygen and iron [25]. This family also includes ubiquitously transcribed tetratricopeptide repeat on chromosome X (UTX-1) and JMJD3, which demethylate H3K27me2 and H3K27me3, respectively. At active enhancers, the function of demethylation and acetylation of H3K27 is likely coupled. This is suggested by observations that depletion of UTX-1 without changing the level of the H3K27 acetyl transferase CREB-binding protein (CBP), which acetylates histone H3 lysine 27 (H3K27ac), leads to a decrease in H3K27ac. By contrast, overexpression of UTX-1 increased H3K27ac [26].

The *de novo* production of ROS during these essential demethylation events appears to be evolutionary accidents that require neutralisation by efficient repair of ROS-induced DNA damage. This becomes particularly important at sites where high concentration of ROS is predicted such as at enhancers and promoters. In favour of this idea, the repair proteins PARP1 and APE1 were found in the chromatin-bound immunoprecipitates of LSD1 [7]. Moreover, NuMA, which is required for oxidative repair at enhancers and promoters, binds JMJD3 [27]. This is interesting because the transcription of *ENNP2*, which encodes autotaxin that is overexpressed in chronic inflammation, is controlled by JMJD3 [27], and NuMA also controls the expression of several proinflammatory genes [18]. Whether histone demethylation is therefore functionally and spatially coupled with DNA repair at gene regulatory regions remains unclear, but highly likely (Figure 1A).

Topoisomerase I activity

The removal of topological constrains during gene transcription requires the action of DNA topoisomerases [28,29]. During this process, DNA strand breaks are formed as part of the topoisomerase catalytic cycle and called cleavage complex intermediates. They are transient reversible breaks that are normally rejoined by the topoisomerase at the end of the catalytic cycle. However, the nearby presence of oxidative breaks, AP-sites and intermediates of incomplete cytosine demethylation can prevent religation and thus require an intervention by DNA repair [30].

Recent models suggest that topoisomerase I (TOP1) is recruited to promoters but does not initiate cleavage until RNA polymerase II (Pol2) is phosphorylated at serine-5 [31]. The phosphorylation is mediated by the chromatin regulator bromodomain-containing protein 4 (BRD4), which binds both TOP1 and Pol2. This molecular switch appears important for releasing paused Pol2 molecules from promoter proximal pause sites to productive elongation [31]. TOP1 also binds other proteins regulating the release of Pol2 from pausing, such as NuMA [18]. However, the role of TOP1 activity and whether (and how) this is regulated by NuMA is unclear. Notably, NuMA depletion or TOP1 inhibition suppresses the transcription of inflammatory mediators including those involved in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections [18,32], suggesting a common mechanism by which transcription is regulated.

The enhancer–promoter interactions and chromatin looping causes topological constraints, which need to be resolved by TOP1 [33]. This increases the chances of endogenous DNA breaks and the demand for repair. The repair of topoisomerase–DNA breaks requires removing the topoisomerase by an error-prone nucleolytic cleavage of DNA or error-free hydrolytic cleavage of the bond linking the topoisomerase to DNA [29,34]. The prototype for the latter activity is TDPs [35,36]. In addition to its role in cleaving AP-sites, the primary job for TDP1 is the liberation of DNA from the abortive TOP1–DNA protein crosslinks. This is initiated via proteolytic degradation of TOP1 to a small peptide, by the small ubiquitin-like modifier (SUMO)-targeted ubiquitin ligase

RNF4, spartan and the cullin pathway [37,38]. The removal of TOP1 peptide is then performed by TDP1, which is regulated by PARP1 and ataxia-telangiectasia mutated (ATM), followed by restoring the DNA termini and ligation (Figure 1B) [39]. Accumulation of TOP1–DNA breaks has been associated with autism [40], spinocerebellar ataxia with axonal neuropathy (SCAN1, caused by mutation in *TDP1*) and ataxia telangiectasia (caused by ATM deficiency) [35,41–43]. It is, however, unclear whether aberrant TOP1 activity affects the repair of oxidative DNA breaks at non-coding regions and how these effects might impact the early stages of transcription.

DNA breaks at non-coding regions and gene transcription: the role of NuMA

Unrepaired oxidative DNA breaks at promoters and enhancers delay the release of Pol2 molecules from pause sites and increase the pausing index (the proportion of Pol2 molecules at promoters compared with gene bodies) [18]. These observations indicate that DNA damage at gene regulatory regions needs to be promptly repaired to support transcription. This inevitably would generate a 'competition' between repair and transcription machines, which needs to be resolved or coordinated. A new player in this coordination is NuMA, which specifically interacts with the initiating and promoter-paused p-ser5 Pol2 and proteins required for the repair of oxidative breaks such as TDP1 and XRCC1 [18].

The role of NuMA in coordinating repair and transcription is regulated by ADP-ribosylation. In terms of repair, the binding of TDP1 and XRCC1 to NuMA is dependent on PARP1 and the recruitment of TDP1 to damaged chromatin is facilitated by NuMA in a PARP1-dependent manner [18]. For transcription, NuMA regulates Pol2 availability at promoters/enhancers by regulating the extent of Pol2 modification by ADP-ribosylation. Loss of NuMA increased Pol2 poly-ADP-ribosylation (PARylation) and chromatin dissociation and decreased its availability at promoters. However, it is currently unknown how NuMA decreases Pol2 PARylation.

The C-terminal domain of NuMA contains 15 out of the 16 reported PARylation sites that were identified in a mass spectrometry screen [44]. It is also the C-terminal domain of NuMA that is sufficient and necessary for repairing oxidative damage [18]. Thus, it is possible that NuMA PARylation increases the concentration of repair machines at regulatory regions and at the same time limits Pol2 PARylation to ensure its availability to support transcription once the damage is repaired. By doing this, NuMA would ensure that repair and transcription machines work in a cooperative rather than a competitive manner for 'optimal' gene expression (Figure 2).

The effect of NuMA in regulating transcription is most pronounced at a subset of protein-coding genes, which includes immediate-early response and proinflammatory genes. Why NuMA loss

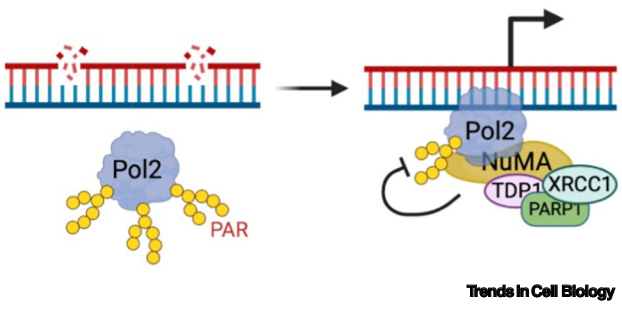

Figure 2. Nuclear mitotic apparatus (NuMA) regulates RNA polymerase II (Pol2) availability for optimal gene expression. Upon DNA damage, Pol2 poly-ADP-ribosylation (PARylation; the product of poly-ADP-ribose polymerase 1 (PARP1) activity) modifies histones, DNA repair proteins and Pol2. NuMA increases the concentration of DNA repair proteins such as tyrosyl DNA phosphodiesterase 1 (TDP1) and limits the PARylation of Pol2, thereby increasing its availability for transcription. Thus, NuMA helps repair and transcription machines to work in a cooperative rather than a competitive manner. Abbreviation: XRCC1, X-ray repair cross-complementing protein 1.

leads to increased DNA damage that is most pronounced at some but not all genes is unclear. ChIP-seq shows that NuMA preferentially binds the promoters of these genes but the reason for this remains unknown [18]. It could reflect differences in DNA sequences, genomic contexts or more vulnerability of these regions to DNA damage.

The recently reported roles of NuMA in nuclear gene transcription is consistent with its reported role in nucleolar gene transcription. NuMA was observed in the nucleolus and shown to interact with RNA polymerase I and ribosomal proteins RPL26 and RPL24 [45]. Interestingly, NuMA localises to ribosomal DNA promoter regions in a manner reminiscent of its localisation at the promoters of nuclear genes. Whether NuMA also promotes the repair of DNA breaks at ribosomal DNA and whether PARylation regulates the function of NuMA during ribosomal DNA transcription are unknown.

One protein, two functions: the role of NuMA in mitosis and DNA repair

NuMA binds dynein and dynactin, which stabilises the microtubule–centrosome interaction during mitosis [46,47]. Two key post-translational modifications have been reported that regulate NuMA function during mitosis. Phosphorylation by cyclin-dependent kinase 1 (CDK1) at T2055 which ensures optimal levels of spindle positioning during metaphase [48–50]. NuMA is also phosphorylated by Aurora-A kinase at S1969, which determines its spindle orientation. The aforementioned two phosphorylation events are distinct from the phosphorylation of NuMA in response to DNA damage. Following oxidative damage induced by ionising radiation, NuMA is phosphorylated at S395, which was first observed in a proteomic screen of proteins phosphorylated on consensus sites recognised by ATM and ataxia telangiectasia and Rad3-related (ATR) [51]. The phosphorylation of NuMA at S395 in response to DNA damage was later confirmed using phospho-specific antibodies and suggested to regulate the chromatin enrichment of DNA repair proteins such as 53BP1 to damaged chromatin [52]. Whether the phosphorylation of NuMA by ATM (or ATR) is required for optimal transcriptional responses and the repair of oxidative breaks at enhancers and promoters is unknown. The protective role of ATM is expected to be highly relevant in neurons since ATM deficiency causes cerebellar neurodegeneration [53] and ATM itself is directly activated by oxidative stress [54].

The other posttranslational modification is PARylation. NuMA was identified as a major acceptor of PARylation by tankyrase 1 in mitosis. Knockdown of tankyrase 1 eliminated NuMA PARylation in mitosis [55]. PARP3 also stimulates NuMA PARylation during mitosis both directly and through tankyrase 1 [56]. In addition to its own PARylation, NuMA binds PARylated proteins, which promotes spindle assembly of exactly two poles during mitotic cell division [57]. In contrast to PARylation by tankyrase and PARP3 during mitosis, NuMA was recently reported to bind PARP1 which plays critical roles in its function in interphase during the repair oxidative DNA breaks [18]. NuMA exists in a complex with Pol2 and oxidative repair factors such as TDP1 and XRCC1, and the presence of PARP1, but not PARP3, is required for complex assembly. Moreover, *in vitro* PARylation assays demonstrated that PARylation increases the binding of NuMA to TDP1. Consistently, the enrichment of TDP1 at damaged chromatin is increased by the presence of NuMA, in a PARP1-dependent manner.

Thus, there appear to be two physically and functionally distinct pools of NuMA, a PARP3-bound pool that has been shown to promote mitotic function and double-strand break repair during cell division, and a PARP1-bound pool that promotes oxidative DNA break repair during gene transcription (Figure 3). The signalling and mechanisms directing the choice of assembling one or the other of these mutually exclusive complexes (NuMA–PARP3 or NuMA–PARP1–TDP1) remain unclear. These molecular decisions are most likely governed by the cell or tissue identity

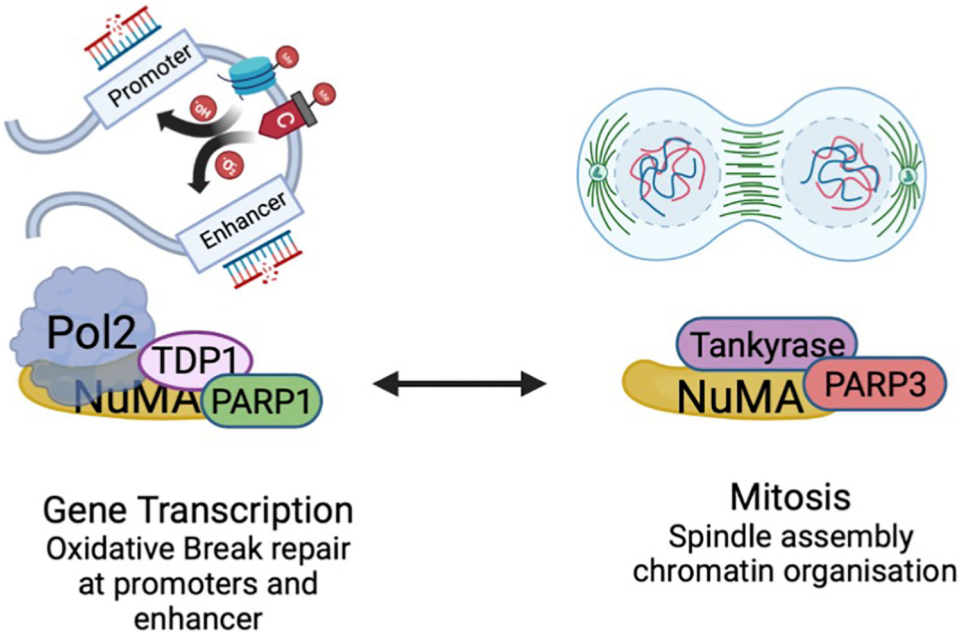

Figure 3. Nuclear mitotic apparatus (NuMA) is involved in two physically and functionally distinct complexes. NuMA binds poly-ADP-ribose polymerase 1 (PARP1) and promotes oxidative DNA break repair at gene regulatory regions, which is required for optimal gene transcription. Another pool of NuMA binds poly-ADP-ribose polymerase 3 (PARP3), which promotes mitotic function and double-strand break repair during cell division. Abbreviations: Pol2, RNA polymerase II; TDP1, tyrosyl DNA phosphodiesterase 1.

(e.g., cycling versus non-cycling) and the nature of stress (oxidative, single-strand breaks or double-strand breaks).

The observation that NuMA limits the PARylation of Pol2 and increases its availability for pause release could be extended to 'buffering' the extent of PARylation necessary for the faithful completion of repair at regulatory regions. If PARylation is not tightly regulated, it could lead to the cytotoxic trapping of PARP1 itself by DNA repair intermediates, which has been shown to be a pathological factor contributing to neuronal dysfunction [58].

The cytotoxic trapping of PARP1 on chromatin has been reported in cells deficient for the single-strand break repair scaffold XRCC1. This trapping promotes the excessive activity of the ubiquitin-specific protease USP3 [14]. USP3 activity reduces the global levels of monoubiquitinated histones important for transcriptional activation, such as histone H2B monoubiquitination at K120 (H2BK120ub). NuMA depletion decreased H2BK120ub, which is associated with reduced transcription recovery from oxidative damage. Whether this effect is due to increased PARP1 trapping on chromatin and increased USP3 occupancy is unknown. Of note, USP3 was not among the proteins whose expression is decreased by NuMA loss, which raises the possibility that NuMA may 'exclude' or compete out USP3 from chromatin to maintain physiological levels of transcription (Figure 4).

The role of oxidative damage at non-coding regions in disease and ageing

Defect in the coordination of transcription and repair is most likely a key cause of age-related and disease-driven neurodegeneration. The direct link between the role of NuMA in sanitising DNA breaks at regulatory regions and disease remains to be established. The analyses of the

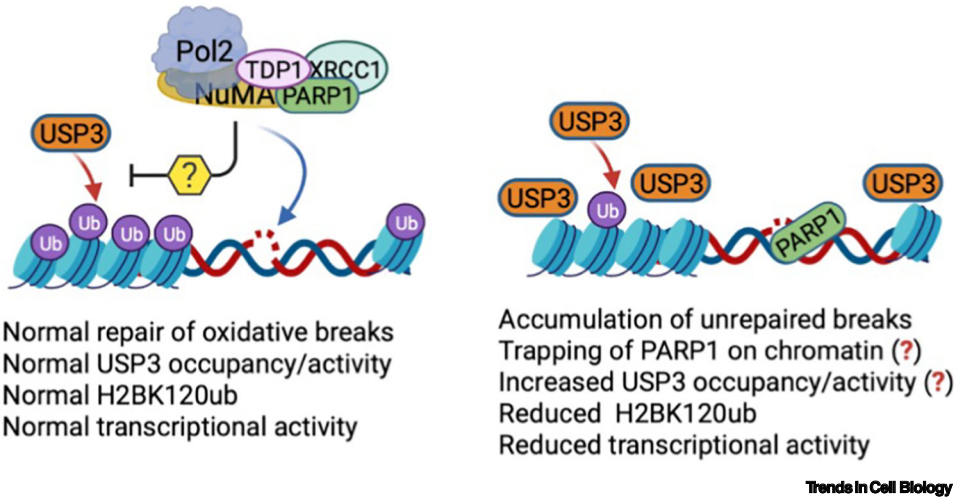

Figure 4. Nuclear mitotic apparatus (NuMA) regulates histone monoubiquitination. Loss of NuMA decreased H2B monoubiquitination at K120 (H2B K120ub), which is associated with reduced transcription recovery from oxidative damage. NuMA may control the chromatin accessibility of histone deubiquitinases [e.g., ubiquitin-specific protease 3 (USP3)] that have been shown to reduce global levels of monoubiquitinated histones important for transcriptional activation. Abbreviations: PARP, poly-ADP-ribose polymerase; Pol2, RNA polymerase II; TDP1, tyrosyl DNA phosphodiesterase 1; Ub, ubiquitin; XRCC1, X-ray repair cross-complementing protein 1.

appropriate animal models and whole-genome sequencing should give more insights into the role of repair at regulatory regions.

The repeated iterations of 5mC oxidation and the associated DNA repair can be tolerated over the lifetime of tissues if the capacity of DNA repair remains intact over time. However, this is unlikely during the ageing process, which could be detrimental because DNA repair polymerases have lower fidelity than DNA replication polymerase. Indeed, increased DNA damage at regulatory regions was first suggested in 2004 by the observation that oxidative DNA damage is increased in the promoters of genes that exhibit reduced expression in the ageing brain [59]. The promoters of the same genes were selectively damaged by oxidative stress in cultured neurons. These early observations are consistent with the idea that DNA damage at regulatory regions may reduce the expression of some genes that are more vulnerable than others, which may contribute to the ageing process. This would also increase somatic mutations by age in non-cycling tissues and clonally select for mutations in cycling cells that could provide a selective growth advantage.

It is worth noting that the neuroprotective effect of XRCC1 has been proposed despite the lack of a direct association between XRCC1 deficiency and neurodegeneration. This is because XRCC1, like NuMA, is an essential protein and germline deficiency causes embryonic lethality. Only recently, a hypomorphic *XRCC1* mutation that causes reduced expression has been linked to neurodegeneration [60]. Whether hypomorphic mutations of *NuMA* are linked to neurological disorders remains to be determined.

Deficiency of XRCC1, ATM, or TDP1 causes neurodegeneration in humans [41,60,61]. The clinical manifestations are attributed to the well-established role of these repair factors at gene bodies. However, the same protein-coding mutations often cause a range of disease onset and severity, which raises the possibility that repair defects and mutations at non-coding gene regulatory regions may modify disease risk, onset or severity. In favour of this idea, suppressing a potential source of breaks at gene regulatory regions by the removal of TET2 offered

neuroprotection in a mouse model of neurodegeneration [62]. It is also consistent with the high mutation rate in neurons in patients with neurodegenerative diseases associated with dysfunctional DNA repair and age-related neurodegenerative disorders [63–65].

Moreover, somatic variants at enhancers have been linked to neurological disorders. For example, Parkinson's disease-associated variants have been identified to regulate the expression of an enhancer RNA in dopaminergic neurons [66]. A microglia gene network was identified in Alzheimer's disease (AD), which was largely confined to microglia enhancers [67]. Mutational signatures reflecting oxidative DNA damage are increased in brain tissues from patients with AD [68–71]. It is therefore plausible to use the specific signature mutations at promoters/enhancers as biomarkers for the early detection/prediction of diseases such as dementia and AD. However, for this to be realised, the ability to detect mutations in an accessible biological fluid such as the blood is required. It remains to be determined if this is feasible and whether it could reflect mutations in the brain or mutations that provide selective advantage of blood cell expansions, which increases the risk of age-associated disease [72].

Outstanding questions

What are the sources of oxidative DNA breaks at regulatory regions? Are intermediates of DNA demethylation the only source?

How does NuMA help repair and transcription to work cooperatively and not competitively?

How does NuMA regulate RNA Pol2 pause release and enhancer activity?

Why do the same protein-coding mutations in several genetic disorders cause a diverse range of disease severity and onset?

Does the burden of oxidative DNA breaks at regulatory regions increase in ageing and in disease? Is it affected by the environment, diet, exercise and lifestyle?

Concluding remarks

The recent advances in exploring the sources and mechanisms of repairing oxidative DNA breaks have shed light into the role of demethylation and NuMA in controlling the integrity of enhancers and promoters. The role of NuMA and the extent by which oxidative breaks at non-coding regions contribute to disease require further studies that move away from focusing on a single cell type or tissue (see Outstanding questions). Understanding whether and how unrepaired DNA damage and mutations in non-coding regions contribute to disease could explain why the same protein-coding mutations cause a diverse range of disease severity and onset. A major challenge here is understanding how pathogenic variants in non-coding regions modify disease risk, onset or severity. The ultimate goal is to improve early diagnosis and deploy better therapeutic strategies [e.g., clustered regularly interspaced short palindromic repeats (CRISPR) based] to modify the function of regulatory regions, genetically or epigenetically. This approach avoids the potential pleiotropic effects associated with drugs that target proteins.

Acknowledgments

I thank our collaborators and members of the laboratory for useful discussions, and apologise to colleagues whose primary work has not been cited due to space limitations. This work was funded by a Wellcome Trust Investigator Award (103844) and a Lister Institute of Preventative Medicine Fellowship (137661).

Declaration of interests

The author declares no competing interests.

References

1. Smith, Z.D. and Meissner, A. (2013) DNA methylation: roles in mammalian development. *Nat. Rev. Genet.* 14, 204–220
2. Sharif, J. *et al.* (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450, 908–912
3. Wu, X. and Zhang, Y. (2017) TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat. Rev. Genet.* 18, 517–534
4. He, Y.-F. *et al.* (2011) Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* 333, 1303–1307
5. Weber, A.R. *et al.* (2016) Biochemical reconstitution of TET1–TDG–BER-dependent active DNA demethylation reveals a highly coordinated mechanism. *Nat. Commun.* 7, 10806
6. Afanas'ev, I. (2015) Mechanisms of superoxide signaling in epigenetic processes: relation to aging and cancer. *Aging Dis.* 6, 216
7. Sengupta, S. *et al.* (2020) Ligand-induced gene activation is associated with oxidative genome damage whose repair is required for transcription. *Proc. Natl. Acad. Sci. U. S. A.* 117, 22183–22192
8. Burrows, C.J. and Muller, J.G. (1998) Oxidative nucleobase modifications leading to strand scission. *Chem. Rev.* 98, 1109–1152
9. David, S.S. *et al.* (2007) Base-excision repair of oxidative DNA damage. *Nature* 447, 941–950
10. Wang, W. *et al.* (2018) Structural basis of transcriptional stalling and bypass of abasic DNA lesion by RNA polymerase II. *Proc. Natl. Acad. Sci. U. S. A.* 115, E2538–E2545
11. Choi, J.-Y. *et al.* (2010) Translesion synthesis across abasic lesions by human B-family and Y-family DNA polymerases α, δ, η, ι, κ, and REV1. *J. Mol. Biol.* 404, 34–44
12. Lebedeva, N.A. *et al.* (2011) AP-site cleavage activity of tyrosyl-DNA phosphodiesterase 1. *FEBS Lett.* 585, 683–686

13. Wei, X. *et al.* (2022) Human TDP1, APE1 and TREX1 repair 3′-DNA–peptide/protein cross-links arising from abasic sites *in vitro*. *Nucleic Acids Res.* 50, 3638–3657
14. Demin, A.A. *et al.* (2021) XRCC1 prevents toxic PARP1 trapping during DNA base excision repair. *Mol. Cell* 81, 3018–3030.e5
15. Mol, C.D. *et al.* (2000) DNA-bound structures and mutants reveal abasic DNA binding by APE1 DNA repair and coordination. *Nature* 403, 451–456
16. Caffrey, P.J. and Delaney, S. (2019) Chromatin and other obstacles to base excision repair: potential roles in carcinogenesis. *Mutagenesis* 35, 39–50
17. Poetsch, A.R. *et al.* (2018) Genomic landscape of oxidative DNA damage and repair reveals regioselective protection from mutagenesis. *Genome Biol.* 19, 215
18. Ray, S. *et al.* (2022) A mechanism for oxidative damage repair at gene regulatory elements. *Nature* 609, 1038–1047
19. Reid, D.A. *et al.* (2021) Incorporation of a nucleoside analog maps genome repair sites in postmitotic human neurons. *Science* 372, 91–94
20. Wu, W. *et al.* (2021) Neuronal enhancers are hotspots for DNA single-strand break repair. *Nature* 593, 440–444
21. Kriaucionis, S. and Heintz, N. (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* 324, 929–930
22. Wang, D. *et al.* (2022) Active DNA demethylation promotes cell fate specification and the DNA damage response. *Science* 378, 983–989
23. Harrison, L.J. and Bose, D. (2022) Enhancer RNAs step forward: new insights into enhancer function. *Development* 149, dev200398
24. Hosseini, A. and Minucci, S. (2017) A comprehensive review of lysine-specific demethylase 1 and its roles in cancer. *Epigenomics-UK* 9, 1123–1142
25. Kooistra, S.M. and Helin, K. (2012) Molecular mechanisms and potential functions of histone demethylases. *Nat. Rev. Mol. Cell Biol.* 13, 297–311
26. Tie, F. *et al.* (2012) Histone demethylase UTX and chromatin remodeler BRM bind directly to CBP and modulate acetylation of histone H3 lysine 27. *Mol. Cell. Biol.* 32, 2323–2334
27. Argaud, D. *et al.* (2019) Enhancer-mediated enrichment of interacting JMJD3–DDX21 to ENPP2 locus prevents R-loop formation and promotes transcription. *Nucleic Acids Res.* 47, 8424–8438
28. Pommier, Y. *et al.* (2022) Human topoisomerases and their roles in genome stability and organization. *Nat. Rev. Mol. Cell Biol.* 23, 407–427
29. Ashour, M.E. *et al.* (2015) Topoisomerase-mediated chromosomal break repair: an emerging player in many games. *Nat. Rev. Cancer* 15, 137–151
30. Pourquier, P. *et al.* (1997) Trapping of mammalian topoisomerase I and recombinations induced by damaged DNA containing nicks or gaps. Importance of DNA end phosphorylation and camptothecin effects. *J. Biol. Chem.* 272, 26441–26447
31. Baranello, L. *et al.* (2016) RNA polymerase II regulates topoisomerase 1 activity to favor efficient transcription. *Cell* 165, 1–16
32. Ho, J.S.Y. *et al.* (2021) TOP1 inhibition therapy protects against SARS-CoV-2-induced lethal inflammation. *Cell* 184, 2618–2632.e17
33. Davidson, I.F. and Peters, J.-M. (2021) Genome folding through loop extrusion by SMC complexes. *Nat. Rev. Mol. Cell Biol.* 22, 445–464
34. Pommier, Y. *et al.* (2016) Roles of eukaryotic topoisomerases in transcription, replication and genomic stability. *Nat. Rev. Mol. Cell Biol.* 17, 703–721
35. El-Khamisy, S.F. *et al.* (2005) Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. *Nature* 434, 108–113
36. Ledesma, F.C. *et al.* (2009) A human 5′-tyrosyl DNA phosphodiesterase that repairs topoisomerase-mediated DNA damage. *Nature* 461, 674–678
37. Sun, Y. *et al.* (2020) A conserved SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-mediated proteasomal degradation. *Sci. Adv.* 6, eaba6290
38. Kerzendorfer, C. *et al.* (2010) Mutations in Cullin 4B result in a human syndrome associated with increased camptothecin-induced topoisomerase I-dependent DNA breaks. *Hum. Mol. Genet.* 19, 1324–1334
39. Das, B.B. *et al.* (2014) PARP1-TDP1 coupling for the repair of topoisomerase I-induced DNA damage. *Nucleic Acids Res.* 42, 4435–4449
40. King, I.F. *et al.* (2013) Topoisomerases facilitate transcription of long genes linked to autism. *Nature* 501, 58–62
41. Takashima, H. *et al.* (2002) Mutation of TDP1, encoding a topoisomerase I–dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. *Nat. Genet.* 32, 267–272
42. Alagoz, M. *et al.* (2013) ATM deficiency results in accumulation of DNA-topoisomerase I covalent intermediates in neural cells. *PLoS One* 8, e58239
43. Katyal, S. *et al.* (2014) Aberrant topoisomerase-1 DNA lesions are pathogenic in neurodegenerative genome instability syndromes. *Nat. Neurosci.* 17, 813–821
44. Hendriks, I.A. *et al.* (2019) An advanced strategy for comprehensive profiling of ADP-ribosylation sites using mass spectrometry-based proteomics. *Mol. Cell. Proteomics* 18, 1010–1026
45. Jayaraman, S. *et al.* (2017) The nuclear mitotic apparatus protein NuMA controls rDNA transcription and mediates the nucleolar stress response in a p53-independent manner. *Nucleic Acids Res.* 45, 11725–11742
46. Merdes, A. *et al.* (1996) A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. *Cell* 87, 447–458
47. Elting, M.W. *et al.* (2014) Force on spindle microtubule minus ends moves chromosomes. *J. Cell Biol.* 206, 245–256
48. Gehmlich, K. *et al.* (2004) Cyclin B degradation leads to NuMA release from dynein/dynactin and from spindle poles. *EMBO Rep.* 5, 97–103
49. Kotak, S. *et al.* (2013) NuMA phosphorylation by CDK1 couples mitotic progression with cortical dynein function. *EMBO J.* 32, 2517–2529
50. Zheng, Z. *et al.* (2014) Cell cycle–regulated membrane binding of NuMA contributes to efficient anaphase chromosome separation. *Mol. Biol. Cell* 25, 606–619
51. Matsuoka, S. *et al.* (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166
52. Salvador-Moreno, N. *et al.* (2019) The nuclear structural protein NuMA is a negative regulator of 53BP1 in DNA double-strand break repair. *Nucleic Acids Res.* 47, 2703–2715
53. Shiloh, Y. (2020) The cerebellar degeneration in ataxia-telangiectasia: a case for genome instability. *DNA Repair* 95, 102950
54. Guo, Z. *et al.* (2010) ATM activation in the presence of oxidative stress. *Cell Cycle* 9, 4805–4811
55. Chang, W. *et al.* (2005) NuMA is a major acceptor of poly(ADP-ribosyl)ation by tankyrase 1 in mitosis. *Biochem. J.* 391, 177–184
56. Boehler, C. *et al.* (2011) Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression. *Proc. Natl. Acad. Sci. U. S. A.* 108, 2783–2788
57. Chang, P. *et al.* (2009) Interaction between Poly(ADP-ribose) and NuMA contributes to mitotic spindle pole assembly. *Mol. Biol. Cell* 20, 4575–4585
58. Adamowicz, M. *et al.* (2021) XRCC1 protects transcription from toxic PARP1 activity during DNA base excision repair. *Nat. Cell Biol.* 23, 1287–1298
59. Lu, T. *et al.* (2004) Gene regulation and DNA damage in the ageing human brain. *Nature* 429, 883–891
60. Hoch, N.C. *et al.* (2017) XRCC1 mutation is associated with PARP1 hyperactivation and cerebellar ataxia. *Nature* 541, 87–91
61. Biton, S. *et al.* (2008) The neurological phenotype of ataxia-telangiectasia: solving a persistent puzzle. *DNA Repair* 7, 1028–1038
62. Marshall, L.L. *et al.* (2020) Epigenomic analysis of Parkinson's disease neurons identifies Tet2 loss as neuroprotective. *Nat. Neurosci.* 23, 1203–1214
63. McKinnon, P.J. (2017) Genome integrity and disease prevention in the nervous system. *Gene Dev.* 31, 1180–1194
64. Abascal, F. *et al.* (2021) Somatic mutation landscapes at single-molecule resolution. *Nature* 593, 405–410
65. Lodato, M.A. *et al.* (2018) Aging and neurodegeneration are associated with increased mutations in single human neurons. *Science* 359, 555–559
66. Dong, X. *et al.* (2018) Enhancers active in dopamine neurons are a primary link between genetic variation and neuropsychiatric disease. *Nat. Neurosci.* 21, 1482–1492

67. Nott, A. *et al.* (2019) Brain cell type–specific enhancer–promoter interactome maps and disease-risk association. *Science* 366, 1134–1139
68. Chun, H. *et al.* (2020) Severe reactive astrocytes precipitate pathological hallmarks of Alzheimer's disease via H_2O_2 production. *Nat. Neurosci.* 23, 1555–1566
69. Wang, J. *et al.* (2005) Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.* 93, 953–962
70. Mecocci, P. *et al.* (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.* 36, 747–751
71. Park, J.S. *et al.* (2019) Brain somatic mutations observed in Alzheimer's disease associated with aging and dysregulation of tau phosphorylation. *Nat. Commun.* 10, 3090
72. Mitchell, E. *et al.* (2022) Clonal dynamics of haematopoiesis across the human lifespan. *Nature* 606, 343–350