Computational and Structural Biotechnology Journal 18 (2020) 207–219

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Review

The genomics of oxidative DNA damage, repair, and resulting mutagenesis

Anna R. Poetsch

St. Anna Children's Cancer Research Institute, Zimmermannplatz 10, 1090 Vienna, Austria

ARTICLE INFO

Article history:
Received 1 October 2019
Received in revised form 13 December 2019
Accepted 21 December 2019
Available online 07 January 2020

Keywords:
8-oxo-7,8-dihydroguanine
8-oxoG
AP site
Genomics
DNA damage
BER
Epigenetics
Oxidative stress
ROS
Mutagenesis
Cancer

ABSTRACT

Reactive oxygen species are a constant threat to DNA as they modify bases with the risk of disrupting genome function, inducing genome instability and mutation. Such risks are due to primary oxidative DNA damage and also mediated by the repair process. This leads to a delicate decision process for the cell as to whether to repair a damaged base at a specific genomic location or better leave it unrepaired. Persistent DNA damage can disrupt genome function, but on the other hand it can also contribute to gene regulation by serving as an epigenetic mark. When such processes are out of balance, pathophysiological conditions could get accelerated, because oxidative DNA damage and resulting mutagenic processes are tightly linked to ageing, inflammation, and the development of multiple age-related diseases, such as cancer and neurodegenerative disorders.

Recent technological advancements and novel data analysis strategies have revealed that oxidative DNA damage, its repair, and related mutations distribute heterogeneously over the genome at multiple levels of resolution. The involved mechanisms act in the context of genome sequence, in interaction with genome function and chromatin.

This review addresses what we currently know about the genome distribution of oxidative DNA damage, repair intermediates, and mutations. It will specifically focus on the various methodologies to measure oxidative DNA damage distribution and discuss the mechanistic conclusions derived from the different approaches. It will also address the consequences of oxidative DNA damage, specifically how it gives rise to mutations, genome instability, and how it can act as an epigenetic mark.

Contents

E-mail address: arpoetsch@gmail.com

https://doi.org/10.1016/j.csbj.2019.12.013

1. Introduction

Oxidative DNA damage is a constant challenge of the genome, arising from exposure to reactive oxygen species (ROS). These can come from multiple external and internal sources, including but not limited to endogenous chemical processes through the cells' own metabolism and enzymatic activity [1], inflammatory processes [2,3], toxins [4], or ionizing radiation [5–7]. When persistent, damage harbors the risk of disrupting cellular function and causing mutation. To cope with this, living organisms have evolved very efficient repair mechanisms [1]. However, these pathways operate not without risks. This is because repair intermediates may increase genome instability and provide indirect routes to disruption of genome function and mutation. Therefore, it is a delicate decision for the cell to balance out whether to start the repair process of a particular oxidative DNA damage site or to leave it unrepaired.

Oxidative DNA damage is among the main mutagenic processes in the germline and accompanies early development [8–10]. Therefore, harmful mutations can potentially be passed on to the majority of cells in the body [11] and the damaged sites can also impact embryonic development. Throughout life, oxidative DNA damage and the associated mutations contribute to the ageing process [12] and the development of age-related diseases [13] such as neurodegeneration [14] and cancer [10,11]. Most cancer treatments cause oxidative DNA damage and DNA strand breaks and thus oxidative DNA damage contributes to long-term side effects in cancer survivors.

Therefore unsurprisingly, oxidative DNA damage and its repair have been of high interest for decades, leading to profound knowledge about its biochemistry as a whole [1]. However, thanks to novel approaches using sequencing-based techniques, it has become apparent that oxidative DNA damage [15–20], repair intermediates [17], and related mutagenesis [17,21,22] are distributed heterogeneously over the genome at multiple levels of resolution. Heterogenous distribution adds to the above-mentioned processes and mechanisms an additional dimension, because it suggests that functional genomic processes impact on oxidative DNA damage distribution and repair specificity. In addition, oxidative DNA damage itself has been identified to take an active role as a site-specific gene regulator and impacts on functional genomics dependent on its genomic location [23–30]. This functional role may come as a double-edged sword due to its side effects on mutagenesis and additional genome instability.

There are now several datasets published that address oxidative DNA damage and repair genome-wide in human [17,18,31], mouse [15,20], and yeast [16], using various conditions and methodology. Increasing amounts of re-sequencing data from tumors [32,33] and healthy tissues [34–37], as well as novel computational methods to extract biological information from mutation data, contribute to the understanding of how oxidative DNA damage affects mutagenesis.

Due to distinct methodological approaches and perspectives, these studies come to diverging conclusions. This review aims at discussing the different methods and attempts to unite the existing viewpoints regarding the mechanistic insight we have achieved on the genomic distribution of oxidative DNA damage, its repair, and consequences for genome function and the distribution of mutations.

2. Origin and repair of oxidative DNA damage

The best studied base to receive oxidative DNA damage is guanine. Its low oxidation potential [38,39] makes it particularly susceptible to singlet oxygen [40,41], leading to the formation of 8-oxo-7,8-dihydroguanine (8-oxoG; Fig. 1). This reaction is estimated to occur on average about 100 to 500 times in each human cell's genome per day [42]. Oxidization of guanine may also occur in the nucleotide pool [43–46]. 8-oxoG could then be incorporated into DNA during replication.

To repair 8-oxoG and other base modifications, the base excision repair (BER) pathway (Fig. 2) has evolved [47], which Enni Markkanen has reviewed in detail [1]. In short, 8-oxoG is excised by 8-oxoguanine DNA glycosylase (OGG1) leaving an apurinic site (AP site). This first step of repair can be very efficient, with the half-life of 8-oxoG lasting only 11 min [48]. AP sites are then processed further into single strand breaks via backbone incision of

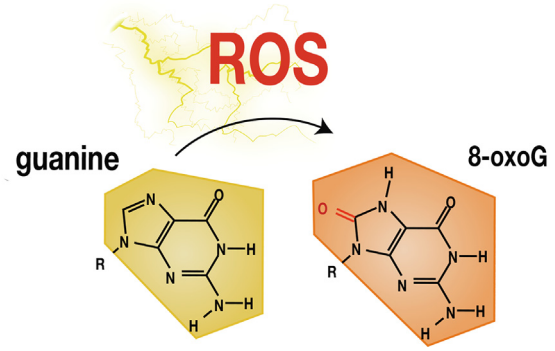

Fig. 1. 8-oxo-7,8-Dihydroguanine (8-oxoG). Under conditions of oxidative stress, 8-oxoG is the result of reactive oxygen species (ROS) modifying a guanine.

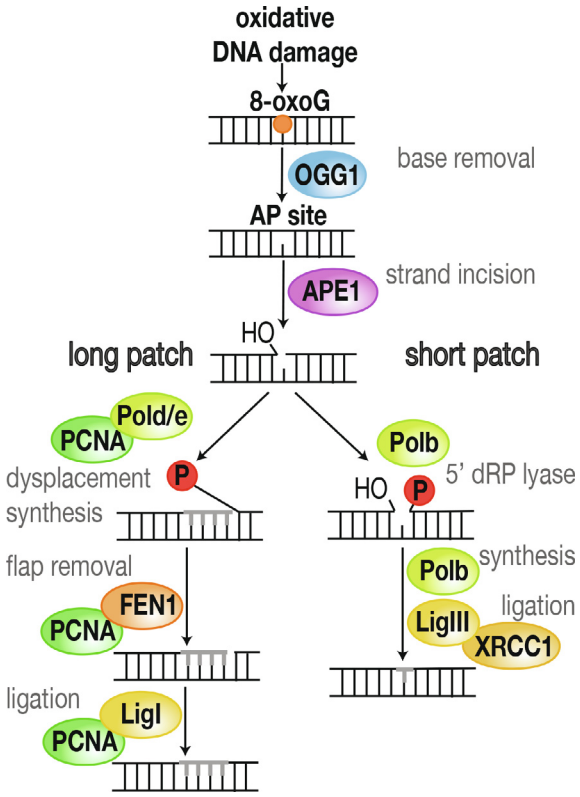

Fig. 2. Base excision repair (BER) of 8-oxo-7,8-dihydroguanine (8-oxoG). Oxidative DNA damage is repaired via several repair intermediates by base excision repair (BER). Through removal of the oxidized base, a reactive apurinic site (AP site) is formed. Incision of the strand creates a single strand break, and the damaged site is then repaired through either short or long patch BER (for details, please see main text).

AP-endonuclease 1 (APE1). In long patch base excision repair, the base and some additional nucleotides are replaced dependent on the activity of polymerase delta (Polδ) and epsilon (Polε) together with proliferating cell nuclear antigen (PCNA). The old strand is removed by Flap-endonuclease 1 (FEN1), before ligase I (LigI) ligates the backbone back together. Short patch base excision repair constitutes of polymerase beta (Polβ) replacing the single missing base, ligase III (LigIII) ligating the DNA backbone back together, and X-ray repair cross-complementing protein 1 (XRCC1) aiding the process and serving as a scaffold for additional factors. The biochemistry of this pathway in general is well understood. However, little is known about its mechanism of action in the context of chromatin and genome function and how this affects different cell types and tissues.

Many base modifications are repaired similarly through BER with various glycosylases initially excising the base. Therefore, AP sites can also be derived from other base modifications as repair intermediates. In addition, AP sites can be the product of spontaneous de-pyrimidination and de-purination. The latter is estimated to occur about 2000–10,000 times in the genome per day [49]. The origin of AP sites is therefore difficult to dissect, if not experimentally controlled. Nonetheless, a substantial part of AP sites are also under physiological conditions derived from oxidative DNA damage and further processing via APE1 is to the best of our knowledge independent of their source.

The interplay of newly emerging DNA lesions versus repair fidelity is reported to result in a steady state of a few thousand 8-oxoG sites [50–52] and ~15,000 to ~30,000 AP sites per cell [52,53]. While these numbers give an idea of the magnitude of damage levels in general, they may not be entirely accurate due to methodological limitations. Also, tissues are exposed differently to oxidative DNA damage due to their location in the body, metabolic activity, and enzymatic processes. Together with differential activity of oxidative stress response [54], DNA repair pathways, and other protective mechanisms, tissues have diverse ways to deal with oxidative DNA damage levels. Therefore, even under physiological conditions, oxidative DNA damage is expected to be highly variable between tissues and cell types, both in absolute numbers, and distribution over the genome. The extent of this variability and the associated regulatory mechanisms are yet poorly understood.

3. Oxidative DNA damage and repair intermediates disrupt cellular function

8-oxoG can lead to disruption of cellular function through multiple mechanisms. First, it can affect how proteins bind to the DNA, as shown for transcription factors. Disruption of their DNA binding can both hinder [23,29,55–58] and promote transcription [20,59–62]. 8-oxoG and AP sites also alter DNA secondary structure, e.g. G-quadruplex folds [63], stacked groups of guanines that affect genome stability, replication, and gene regulation through enforcing single stranded DNA and providing protein binding sites, as reviewed by Kwok et al. [64]. Intact G-quadruplex folding is of particular importance at telomeric repeats, where they contribute to telomere protection. Therefore, oxidative DNA damage at telomeres leads to dysfunctional maintenance effecting telomere length and genome instability [26–28,65]. Changes to secondary structure may also contribute to the interference of base lesion clusters with the replication fork [66], a potential route contributing to additional genome stability.

4. Risks from repair and its intermediates

Converting 8-oxoG into an AP site also harbors risks that may exceed the danger of persisting damage. First, there is a risk of information loss and mutation as described in more detail below. In addition, AP sites have strong effects on DNA secondary structure, protein binding, and G-quadruplex folding [63]. AP sites compromise genomic processes [67], such as stalling transcription and replication [29,30]. The reactive aldehyde group of the AP site may react with amino groups to form DNA-protein crosslinks [68] with potentially deleterious consequences for genome integrity. In addition to AP sites, the related β-elimination product (βE-site), and repair associated conversion to single strand breaks harbor the risk of damage acceleration towards double strand breaks (DSBs) and as a result genome instability, mutation, translocation, and loss of information. Consequently, there is the need for a careful balance between retaining an oxidative DNA damage in the genome versus its repair. This may explain why OGG1 becomes enzymatically inactivated during excessive oxidative stress [69,70]. Moreover, at the point of halted repair, the damage can act as an epigenetic mark as detailed below [59–62].

In summary, different regions in the genome display diverse potential for functional disruption through 8-oxoG and AP sites. The risks are largely dependent on other cellular processes and states, e.g. the cell cycle, replication and transcriptional activity. Decision processes are therefore deeply embedded in other mechanisms of genome regulation and dependent on potential impact of damage and repair. Location of DNA damage levels and their repair intermediates are therefore the first indicator to understand which regions of the genome are protected and the potential mechanisms leading to genomic prioritization of damage prevention and/or repair.

5. Methods to measure genomics of oxidative DNA damage

Several techniques have been developed in recent years to measure oxidative DNA damage and repair intermediates (Fig. 3). Each method comes with unique benefits and pitfalls (Table1). They are all based on enrichment of the damaged DNA through arrays or sequencing, either by pull-down of the damaged DNA, or analogous strategies.

8-oxoG location in the genome was first addressed using antibodies [71], initially at large-scale resolution. Amente et al. adapted this approach for next generation sequencing as OxiDIP-seq [18]. In short, DNA is fragmented into several hundred base pair long fragments, bound by an 8-oxoG specific antibody, and enriched through protein-G-coated magnetic beads. The enriched DNA, as well as the non-enriched input DNA is then prepared for next generation sequencing and the resulting fragments aligned back to the respective genome. Increased sequencing read coverage is used to quantify damage levels genome-wide. Using this method, Amente et al. could detect 8-oxoG with a resolution of several hundred base pairs [18]. In addition to antibody-mediated enrichment, several techniques were developed that enrich for oxidatively damaged DNA using chemical biology.

OG-seq, the method developed by Ding et al. [15] is taking advantage of the propensity of 8-oxoG to become hyperoxidated in response to mild oxidants. Using this chemical property, the damaged base is converted into an acceptor for a biotin tag. After this chemical reaction, the covalently tagged DNA is enriched through a biotin-streptavidine pull-down and subsequently sequenced. The resolution of this method is similar to OxiDip-seq limited only by the size of DNA fragmentation.

In Click-code-seq, developed by Wu et al. [16], the specificity for 8-oxoG is achieved through utilizing the specific glycosylase formamidopyrimidine DNA glycosylase (FpG) to remove the damaged base and APE1 to create a strand break. The single nucleotide gap is then refilled with a Click-tagged guanine. Using this nucleotide as an acceptor for the Click reaction, a code sequence is added to the

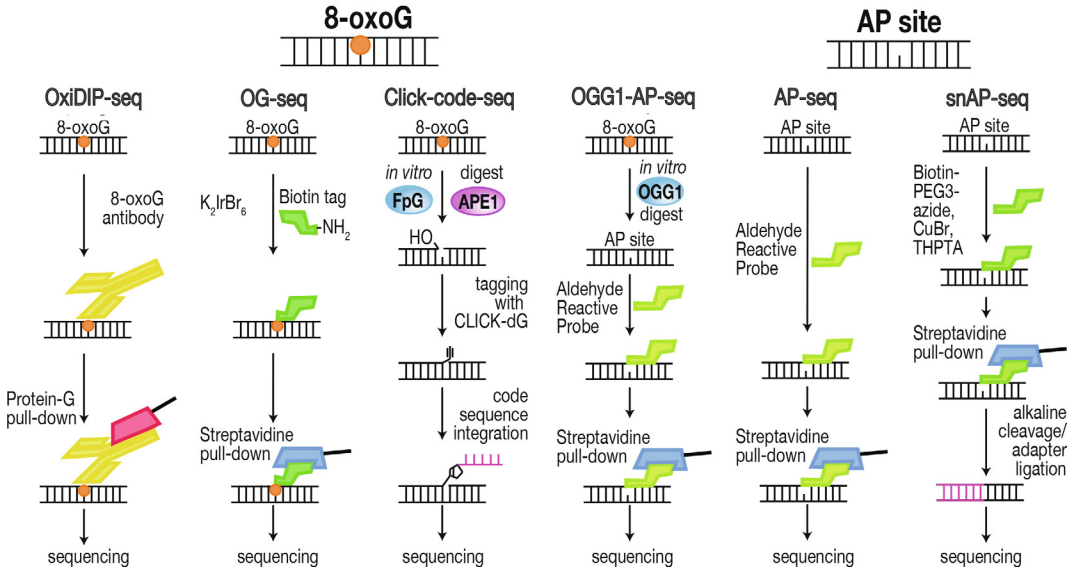

Fig. 3. Methods to measure oxidative DNA damage genome-wide. Several methods have been developed that utilize next generation sequencing to assess the genome wide distribution of 8-oxoG and the repair intermediate AP site. For details on the methods, please see the main text.

Table 1
Properties of the different methods to measure oxidative DNA damage genome wide.

	OxiDIP-seq	OG-seq	Click-code-seq	OGG1-AP-seq	AP-seq	snAP-seq
Damage recognition	✗	✗	✓	✗	✗	✓
Single nucleotide resolution	✗	✓	✓	✓	✓	✓
Potential single nucleotide resolution	✗	✗/✓	✗	✓	✓	✗
Damage recognition before sonication	✓	✓	✓	✓	✓	✓
Ease of use	✓	✓	✓	✓	✓	✓
Reagents commercially available	✓	✓	✗	✓	✓	✗
Potential problems with specificity	General antibody specificity	Induced oxidation and probe side reactions	Specificity of FpG for 8-oxoG and incomplete masking of AP sites/strand breaks	Specificity of OGG1 for 8-oxoG and incomplete masking of AP sites	Side reactions with other aldehydes, e.g. 5-fU, if present	Side reactions with other aldehydes. Incomplete depletion of 5-fU

DNA at the position where the 8-oxoG had been located. This oligonucleotide then serves as an adapter for sequencing. Click-code-seq is so far the only method achieving single-nucleotide resolution. While used to measure 8-oxoG, this method would in principle be applicable to measureing other base adducts through the use of different glycosylases, as well as AP sites.

In addition, our method, so called AP-seq [17,72], can be used to measure AP sites as the first repair intermediate, but indiscriminate of the source of the AP site. To prevent other sources of AP sites, such as spontaneous depurination from confounding the measurements, a careful experimental design that focuses on specific introduction of oxidative DNA damage allows interrogation of early steps of damage processing when compared to preexisting background AP site levels. For AP-seq, AP sites are tagged with biotin using an aldehyde reactive probe (ARP). This probe was developed by Kubo et al. [73] in 1992 and used for a variety of assays over the decades since [74–77]. ARP reacts under the recommended conditions specifically with the aldehyde group of the AP site and consequently introduces a covalent biotin tag into the DNA at the damage site. The DNA is then enriched through a streptavidine pull-down and prepared for sequencing. Masking AP sites with methoxyamine before *in vitro* glycosylation with OGG1 allows for specific detection of 8-oxoG through conversion into a secondary AP site with subsequent use of ARP to enrich the DNA for sequencing (OGG1-AP-seq). Similarly to OxiDIP-seq and OG-seq, resolution of AP-seq and OGG1-AP-seq is determined by the fragmentation size of the genomic DNA.

Finally, snAP-seq was developed as an alternative method to measure AP sites through a different chemistry [31]. AP sites and 5-formyl-uracil (5-fU) are tagged with a hydrazino-*iso*-Pictet-Spengler (HIPS) probe and attached to biotin using Click chemistry. The damaged DNA is then enriched via streptavidin-mediated pull down. Alkaline cleavage is used to achieve single nucleotide resolution and to selectively release AP sites off the beads, while 5-fU containing fragments are retained and therefore depleted using this strategy. Alkaline cleavage would in principle also be applicable for the other chemical biology-based methods to achieve single nucleotide resolution for OG-seq and AP-seq.

Differences between the methods can be found in resolution, specificity, and ease of use. Single nucleotide resolution is so far achieved by Click-code-seq and snAP-seq, whereas the resolution of other methods is determined by the size of enriched DNA fragments, which may be improved by using alkaline cleavage for OG-seq and AP-seq.

Specificity is dependent on the recognition step of the oxidative DNA damage. It may be affected by sequence content, helix properties, and DNA secondary structures.

Unspecific antibody binding is an intrinsic problem affecting all antibody-based methods, but has been especially raised in the context of 8-oxoG measurements [15,78,79]. Specificity issues however also affect the methods based on chemical biology. Both OGG1-AP-seq and Click-code-seq are based on a glycosylation step with the assumption that OGG1 and FpG are specific for 8-oxoG and independent from surrounding sequence and its physicochemical properties. In addition to 8-oxoG, one has to expect glycosylation also for 8-oxoG oxidation products, such as FapyG [80], as well as potential enzymatic activity for other base modifications [81]. Both methods would also detect AP sites and some strand breaks/nicks unless these are completely masked and processed into non-reactive groups.

Furthermore, AP-seq is based on a chemical reaction that may suggest to result in side products with other aldehyde groups, e.g. damaged backbone-ribose and other base oxidization products. Indeed, a similar chemistry has been used to detect 5-formyl cytosine (5-fC) [82] with different reaction conditions. Such conditions would also be permissive for detection of 5-fU. However, the conditions used for AP-seq are not efficient for the detection of 5-fC [82]. AP-seq is therefore not expected to lead to substantial background through 5-fC with the reaction conditions used [17]. Particularly due to 5-fU, it is however advised to carefully control experiments, if they are performed in embryonic stem cells, where both 5-fC and 5-fU naturally occur.

In snAP-seq, where the probe may also react with other aldehydes, side-reactions with 5-fU are compensated through a depletion step, while side reactions with 5-fC are shown also not to occur when using synthetic DNA.

OG-seq does not rely on antibodies or glycosylation. Instead, 8-oxoG is further oxidized to allow tagging with biotin. Here the assumption is that hyperoxidation is efficient, irrespective of sequence context and secondary structures, and that oxidative conditions do not cause additional 8-oxoG. The following biotinylation is assumed to be specific for the hyperoxidised 8-oxoG. As with all other methods mentioned above, there is potential for unintended side reactions, such as reactions of the biotin tag's aminogroup with other sites than the hyperoxidised 8-oxoG.

In summary, all methods have the potential of unspecific enrichment of genomic DNA and unintended effects of sequence content and secondary structures. The diversity of the approaches creates however a valuable resource for cross-validation as the sources for unspecificity differ.

Every approach to measure oxidative DNA damage is facing the challenge of technical oxidative DNA damage that is accumulated during sample processing. While such artifacts cannot be prevented in full [83], the developers of the above mentioned methods have chosen different strategies to reduce technical oxidization reactions. Added anti-oxidants to the reaction conditions include 50 μM N-*tert*-butyl-a-phenylnitron for OxiDIP-seq, 100 mM deferoxamine with 100 mM butylated hydroxytoluene for Click-code-seq and OG-seq, and 20 mM TEMPO for snAP-seq. Additionally, sonication for fragmentation is a source of oxidative DNA damage [84]. For Click-code-seq and OxiDIP-seq it seems unavoidable to perform sonication before 8-oxoG is recognized by the glycosylase and antibody respectively. Also for the 5-fU depletion step in snAP-seq, sonication is necessary to be performed before AP sites and 5-fU are tagged. For AP-seq, sonication is performed after the damage site is covalently tagged with biotin, which would also be an option for OG-seq.

All pull-down-based methods are equal in their ease of use. Whereas OxiDIP-seq in its execution steps is most similar to widely used ChIP-seq, the chemical biology strategies of the other methods only add a few extra steps. For OxiDIP-seq, OG-seq, and AP-seq, all material is commercially available. Click-code-seq relies on Click-tagged guanine and the code sequence oligonucleotides, which were synthesized for the respective study. snAP-seq relies on a custom-made chemical probe.

In conclusion, all methods that have been used to measure oxidative DNA damage genome-wide have advantages and disadvantages. The different strategies may lead to slightly different outcomes, as they may be prone to different side reactions and sources for background. It is therefore important to cross-validate biological findings and to carefully evaluate mechanistic results based on the methods and systems used.

6. The genomic distribution of oxidative DNA damage and repair

Genomic distribution of oxidative DNA damage is a snapshot that reflects the location specific balance of DNA damage impact and the different steps of repair. Both layers can be affected by different modes of selectivity and regulation at different scales of resolution. Before the development of sequencing-based genome-wide methods, measurements on a larger scale used 8-oxoG antibody pull-down experiments paired with microarrays. They show 8-oxoG accumulation in gene deserts, linked to chromosome territories adjacent to perinuclear regions [71,85]. The perinuclear position of heterochromatin [86] may contribute to increased exposure to oxidative stress and therefore increased oxidative DNA damage. Interestingly, such regions tend to be GC poorer than transcriptionally active euchromatin, so the observation of higher 8-oxoG levels leads to the conclusion that direct effects of sequence content in general cannot explain accumulation of oxidative damage in heterochromatin. Phase separation of heterochromatin is likely to affect the accessibility of DNA to reactive oxygen species, as it creates a distinct physicochemical environment [87,88], which may also affect the generation and mobility of ROS and the presence of scavenger molecules. This would however be expected to have a protective effect rather than leading to increased exposure.

Instead, oxidative DNA damage distribution at the level of chromatin domains is dominated by differences in base excision repair activity. Akatsuka et al. show that damage distribution differences by sequence content do not occur under OGG1 deficiency [85]. This is due to the major early base excision repair factors being specifically and rapidly recruited to open chromatin regions upon oxidative DNA damage [89–91]. This specific recruitment is therefore the most likely explanation of increased 8-oxoG accumulation in gene deserts and heterochromatin with the peak of OGG1 recruitment to open chromatin marking the fastest repair kinetics of the damaged genome [90]. Efficient repair in heterochromatin may also require additional DNA damage response, similar to double strand break repair requiring ataxia telangiectasia mutated (ATM) signaling [92]. ATM interactor (ATMIN) could be the possible link to oxidative DNA damage, as it has been described to protect neurological tissue from oxidative DNA damage and contributes to glioblastoma formation [93,94].

At the nucleosome level, accessibility to the DNA repair machinery is a crucial factor. *In vitro,* nucleosomal structure has been shown to inhibit the initial steps of base excision repair and chromatin remodeling is required to efficiently remove damage [95–98]. Therefore, treatment with the deacetylase inhibitor trichostatin A, a drug that opens chromatin, enhances DNA repair efficiency in general [99]. In conclusion, even before sequencing-based genome wide methods, it was established that accessibility for OGG1 and the base excision repair machinery is a major factor that shapes oxidative DNA damage distribution with increased

damage levels in heterochromatin and at sites of tightly packed nucleosomes.

It became possible through the novel sequencing-based methods to investigate functional genome elements at finer resolution. Indeed, 8-oxoG could be confirmed to accumulate at sites of high nucleosome occupancy in yeast [16]. Generally, different types of repeats accumulate large amounts of 8-oxoG, particularly telomeres [16,17] and microsatellites of particular sequence content [15,17]. While the repeated telomeric sequence TTAGGG (human) is rich in guanine stretches and the 5′ guanine particularly prone to oxidation [100,101], the affected sequences in microsatellites, e.g. $(TG)_n$ and $(TGGA)_n$ do not necessarily require rows of Gs. Both on G-quadruplex folds and microsatellites, 8-oxoG accumulation might be connected to DNA secondary structure, possibly leading to higher sensitivity towards base modification or impaired excision by OGG1 as has been shown for some secondary structures at the telomeres [102]. As a potentially compensating mechanism, additional glycosylases can cover 8-oxoG excision at G-quadruplex folds, such as the glycosylases NEIL1 and NEIL3 [103]. Also, it has been observed that 8-oxoG destabilizes G-quadruplexes [104]. On the other hand, G-quadruplex folds can be stabilized through oxidative DNA damage with a conformational change, which is stabilized by converting the 8-oxoG in a 5th G track into an AP site and subsequent APE1 binding [105].

Additional systematic assessment of oxidative DNA damage and its processing on secondary structures in general and particularly in different types of quadruplex folds may help to better explain both the involved regulatory processes and secondary structure-associated mutagenesis [106].

Interestingly, AP sites accumulate at specific locations in the genome when measured with AP-seq in liver cancer cells treated with ionizing radiation [17], while the profile obtained using snAP-seq in Hela cells with and without APE1 silencing resembles background [31]. The reasons for this discrepancy can be manifold and remains to be investigated further. In the AP-seq dataset, AP sites are generally reduced in heterochromatin versus euchromatin [17] and their specific accumulation can be also found in repeats, particularly retrotransposons [17], which have the potential to become activated in response to DNA damage in general and ionizing radiation in particular [107,108].

The role of oxidative DNA damage in regulatory elements of high GC content, such as promoters, enhancers, and coding sequence is controversial. When averaging as a metaprofile over multiple regions for 8-oxoG in yeast [16] and AP sites in human liver cancer cells [17], such regions show a general depletion of oxidative DNA damage. On the other hand, location of 8-oxoG and AP sites was found explicitly at promoters using genome-wide approaches paired with peak calling [15,18,20,31]. This is a contradictory finding only at first sight. Indeed, peak calling should be applied with caution to such data and in GC-rich DNA regions due to the false positive peak-calling rate induced by GC-content-induced sequencing bias of the input sample. However, some specific promoters do indeed accumulate 8-oxoG. These are predominantly promoters that harbor G-quadruplex folds. G-quadruplex folds generally accumulate 8-oxoG, both when located in promoters and elsewhere [17], as has been shown previously on specific promoters, e.g. of *VEGF* [60,63,109].

At the level of coding and regulatory sequence, reduced AP site levels and 8-oxoG are correlating with GC content [17] as well as histone marks of open chromatin and marks of actively transcribed exons [16,17]. In a different study, 8-oxoG is reported to accumulate specifically in the gene body of long transcribed genes [18]. While the authors conclude that increased DNA damage rates may lead to increased 8-oxoG, it was however not excluded that damage may accumulate in repeats and retrotransposons hosted in their introns. However, active transcription and persistent single-strandedness may contribute to increased oxidative DNA damage at actively transcribed sites and is balanced with different repair kinetics, aided by accessibility of the DNA [95–98,110].

In addition, the relation to $H3K36me^3$ is striking. This is the mark of actively used exons and is serving as a guide to exons for mismatch repair, through direct interaction of this complex with the mark [111], which leads to locally reduced mutation rates. This reduction is evened out under dysfunctional mismatch repair [112–114]. Whether base excision repair may be following similar mechanisms, is not yet clear. While not yet investigated for oxidative DNA damage repair, repair of methylation adducts on guanine is preferentially conducted in promoter regions [115].

Other repair pathways, specifically double strand break repair are governed through signals spreading from chromatin architectural loop anchors [116]. Since AP site location also relates to chromatin architecture dependent on the predominant mark of the respective loop [17], reduced oxidative DNA damage patterns in coding sequence may also reflect DNA repair organization that follows a similar seeding principle.

Specific recruitment and regulation of repair on functionally important genome elements and over genes allows balancing risks of damage versus repair and to flexibly prioritize. Such risk management is of particular importance, as these dynamic regions of the genome are particularly prone to strand break formation [117] and there is strong selective pressure of mutation in such areas of the genome [118].

The precise regulatory mechanisms and pathways how functionally relevant areas of the genome are protected are not known, but there are some processes of functional genomics known to interact with BER. First, on the level of 8-oxoG and OGG1, these are cytosine methylation [119–123] and chromatin remodeling (CHD4) [124], transcription coupled repair processes (Cockayne Syndrome A and B; CSA, CSB) [125], poly-ADP-ribose polymerase 1 [126], CUX1 and CUX2 [127,128], and components of the RNA splicing machinery (SNRPF) [129]. Second, on the level of AP sites and APE1, epigenetic modifiers have been shown to influence APE1 location, specifically the SET complex, histone deacetylases (HDACs) and sirtuin1 (SIRT1) [130,131]. Localization of APE1 has been described to be mediated by nucleophosmin (NPM1) [132]. APE1 also interacts with splicing regulators (HNRNPL) [133] and transcription factors (AP1) [134], and is recruited to DNA-RNA hybrids and R-loops [135], providing a link to active transcription.

Finally, regarding direct sequence context, the only method for measuring 8-oxoG with single nucleotide resolution found a bias towards a guanine 3′ to 8-oxoG [16], which can be explained by chemical sequence predisposition [100,101]. Therefore, also dinucleotide distribution over the genome affects the location of 8-oxoG.

In summary, there are multiple pathways as well as DNA sequence characteristics implicated in potentially influencing genome-specificity of oxidative DNA damage and several steps of repair at multiple layers of resolution. Relative contribution of each layer and the precise mechanisms are poorly understood, although a strong determinant of damage distribution can be found in the DNA's accessibility to base excision repair, hindered by tight nucleotide packaging and heterochromatin. Nevertheless, especially in regulatory and coding regions of the genome, interactive contributions are expected to lead to the final distribution of oxidative DNA damage, which is therefore dependent on the relative activity of each factor in a given cell type. Lack of knowledge is largely due to the lack of techniques in the past to study such mechanisms genome-wide and in single cells. Now we do have the means to develop the necessary methodology and to dissect the reasons that underlie the heterogeneous DNA damage distribution.

7. Oxidative DNA damage as an epigenetic mark

The flip side of oxidative DNA damage is a role in the processes of cell fate decision making and differentiation. In this context oxidative DNA damage and its repair intermediates may serve as epigenetic marks. Gene regulatory function is thought to be mediated through several distinct molecular mechanisms, as reviewed in more detail by Wang et al. [62] and Fleming et al. [25]. They are involved in oxidative stress response accompanying differentiation processes in the heart [24] and adipose tissue [20], and become of crucial importance, when OGG1 function is impaired [69,70].

The interaction of 8-oxoG and AP sites with G-quadruplex structures is critical for oxidative DNA damage to epigenetically affect gene activity. While 8-oxoG is potentially destabilizing the formation of G-quadruplex folds, AP sites are known to possibly stabilize them. Proposed modes of action have been described for the *VEGF* promoter, where an AP site stabilizes the quadruplex fold and gene activation is mediated through catalytically inactive APE1 binding [60,109]. G-quadruplex formation enforces single-strandedness of the opposite strand and may also facilitate the assembly of the transcription machinery [64]. A similar mechanism is also described for the endonuclease III like protein 1 (NTHL1) [61]. In addition to the formation of a G-quadruplex, NTHL1 may impact on gene regulation via a Z-DNA/hairpin balance [136]. On the other hand, at the *KRAS* promoter, it is proposed that activation is not mediated by the AP site, but by binding of catalytically inactive OGG1 to 8-oxoG, which prevents the formation of a silencing G-quadruplex fold [104,137]. A large number of promoters that may be regulated in a similar fashion have been identified recently [25] and are currently waiting to be characterized in depth.

Modified DNA has the potential to alter binding of regulatory proteins, including transcription factors. This was shown for some distinct examples, e.g. SP1 [55], p50 [56], and CREB [23]. While a comprehensive assessment has not been performed yet, the strong effect of DNA-methylation on DNA-protein interactions [138] indicates that 8-oxoG and AP sites may also have a substantial impact on the binding of regulatory proteins to DNA. This might be mediated not only through altering the motive, but also through changing surrounding secondary structures. In addition, oxidative DNA damage and accompanying processes can contribute to the mechanisms of the cell sensing oxidative stress. In states of hypoxia, ROS can stimulate transcription by promoting the binding of hypoxia inducible factor 1 (HIF1) to hypoxia response elements (HREs) [59], which occurs at promoters of oxidative stress response genes, such as the vascular growth factor *VEGF*.

Epigenetic function of oxidative DNA damage may also act in concert with the DNA methylation machinery and metabolism of other epigenetic marks. Repair of 8-oxoG is mechanistically linked to DNA methylation [139], but the exact mechanism of how DNA methylation influences oxidative DNA damage repair and vice versa is poorly understood.

Finally, oxidative DNA damage is also a result of specific generation of ROS generation by enzymes of the epigenetic machinery. Indeed, lysine specific demethylase 1 (LSD1), an enzyme that removes methyl groups from histones, produces ROS as a byproduct of its enzymatic function. This type of oxidative stress occurs directly next to the DNA. An effect on gene regulation through this mechanism is shown for estrogen-induced gene expression [140]. The mechanism relies on the catalytic function of OGG1 and topoisomerase IIb-induced structural remodeling at the promoter. Analogous mechanisms might apply to multiple other genes, considering LSD1's frequent activity at various enhancers and promoters across the genome.

In conclusion, the existing mechanistic data suggest that oxidative DNA damage is involved in gene regulation similar to an epigenetic mark and can interact with other epigenetic processes. Why would the cell use a mutagenic mechanism such as oxidative DNA damage for gene regulation? Essentially the same question is applicable to DNA methylation, given the high mutagenic potential of the deamination reactions converting methylated cytosine to thymine [141,142]. How the benefit of epigenetic regulation is balanced with detrimental risks of mutagenesis is a still an unsolved conundrum. This balance is likely regulated in a tissue-specific manner considering also varying mutation tolerance. In this context, the epigenetic role of oxidative DNA damage in differentiation could be of crucial importance, as mutations during development are passed on to descending cell generations [11].

8. Mutagenesis from oxidative DNA damage

Of the about 20,000 base lesions that are estimated to occur on average in cells under physiological conditions [42], most do not progress into mutations because they are repaired or tolerated during replication without loss or change of genetic information. However, unrepaired oxidative DNA damage and repair intermediates harbor risks of mutagenesis. This is mediated through mispairing of 8-oxoG with adenine (Fig. 4), which may occur during DNA replication [143]. However, this mismatch has its own safety mechanism. Dedicated MUTYH is responsible for recognizing and removing the adenine, and correct what otherwise might manifest as a C-to-A mutation [144], such as under conditions of silenced MUTYH or if it is not recognized (Fig. 5). However, C-to-A mutation may also be induced by oxidative DNA damage via an indirect mechanism. Not through mispairing of 8-oxoG, but through loss of information by creating AP sites as a repair intermediate. During replication, AP sites cannot be paired appropriately, typically leading to incorporation of an adenine opposite the gap, following the so-called A-rule [145–147]. The result of both routes is indistinguishably a C-to-A mutation.

In addition, oxidative DNA damage can lead to T-to-G mutations, mediated by 8-oxoG-adenine mismatches from oxidative damage of the nucleotide pool [43–46,148]. T-to-G mutations are particularly found in esophageal adenocarcinoma and inflammatory Barret's esophagus [149–152]. A first line of repair is the recognition of the mismatch by polymerase proofreading, then post-replicative mismatch repair, and – if still persistent – BER.

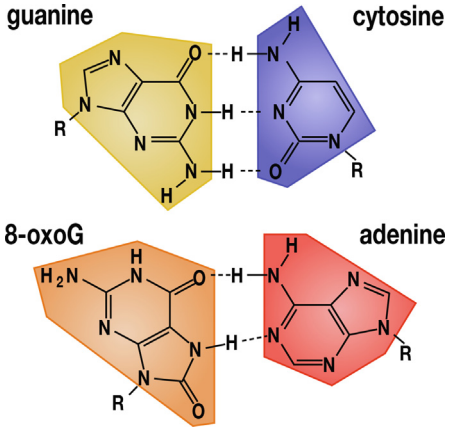

Fig. 4. Route to C-to-A mutation through 8-oxoG-adenine-mismatches. Oxidative DNA damage provides direct routes to mutations. While guanine usually pairs with cytosine, 8-oxo-7,8-dihydroguanine (8-oxoG), the most frequent type of oxidative base damage, may cause mispairing with adenine through a conformational change. This is one route to oxidative DNA damage induced mutations.

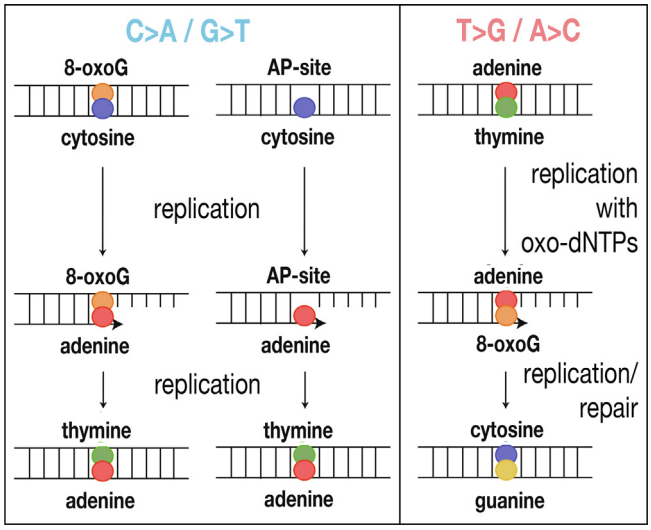

Fig. 5. Routes to oxidative DNA damage dependent mutagenesis. Oxidative DNA damage provides direct and indirect routes to mutagenesis, particular on single nucleotides, i.e. C-to-A and T-to-G and their reverse complements. Mispairing of 8-oxoG with adenine during replication leads to C-to-A mutation through erroneous repair or in the next round of replication. The same mutation can however also be the result of replication encountering an AP site. Following the A-rule, an adenine may be incorporated opposite the AP site, which also leads to C-to-A mutation. Incorporation of 8-oxoG from an oxidized nucleotide pool may also lead to mispairing with adenine. Through erroneous repair or in the next round of replication, the mismatch may lead to T-to-G mutation.

Action of MUTYH would in this case not be preventive of mutation, as it would "repair" the correct base. Also unrepaired 8-oxoG may lead to mutation in the next round of replication through pairing with cytosine, ultimately causing T-to-G mutation.

9. Genome-specificity of mutagenesis

Variability of mutation rates over the genome is now increasingly well understood [21,114,153] due to the large amounts of cancer resequencing data available to study mutagenesis and the development of new algorithms, statistical and computational approaches. One particular challenge is still the differentiation of technical artifacts from oxidative DNA damage-derived mutations that originate from molecular biological processes. Artifacts typically arise from culturing conditions [154], or technical sample processing [84,155,156]. While technical artifacts are still difficult to avoid or differentiate from biological effects, their total impact on a given sample can be assessed for quality control purposes [84].

Variability of mutation distribution over the genome occurs at multiple scales of resolution from the immediate sequence content to megabases as the sum of relative contributions by DNA damage mechanisms, repair pathways, and replication accuracy. The mechanistic details of each layer and how the mutagenic mechanisms act in the context of the genome are still poorly understood for the majority of mechanisms, particularly for those that arise from oxidative DNA damage.

We are however now able to assess what mutagenic mechanisms are active in which systems, aided by the mutational signatures developed by Ludmil Alexandrov, Serena Nik-Zainal and others [157–159]. These are fingerprints of mutations in their trinucleotide context, which can be extracted from cancer genomes using the pattern differentiation algorithm non-negative matrix factorization. The six different options of single nucleotide changes are expanded to 96 different mutation types through including the first preceding and following nucleotides. Co-ocurrence of particular patterns allows for separation of mutagenic mechanisms, which are active in particular cancer samples. Retrospectively, the mutational signatures are linked back to the biological mechanisms [154,160–162], the etiology for most is however still not entirely understood [159,163]. With increasing numbers of sequenced cancer genomes, more mechanisms can be differentiated. They are typically the result of several processes acting simultaneously; DNA damage in interaction with repair [164], replication accuracy [165,166], and specific mutagenic mechanisms of enzymatic origin, such as APOBEC3A [157,167] or error-prone DNA polymerases [168].

Several mechanisms leading to oxidative DNA damage-derived mutations can be separated out through mutational signatures. As an additional feature, they are giving valuable information about the immediate sequence context for the signatures that each mechanism leaves in the genome (Fig. 6).

Signature 18 is thought to be the main ROS associated fingerprint. It mainly consists of C-to-A mutations, commonly preceded or succeeded by an adenine or thymine, similar to the mutation patterns in response to potassium bromate treatment as a source of ROS [154]. It occurs commonly as a background signature throughout various cancer types, but rarely as a dominant mutagenic mechanism. Proportions of this signature can however get quite high in cancers that do not have other major mutagenic mechanisms active, such as neuroblastoma [157]. This mutational signature is also frequently found as an artifact in cell and organoid culture [154]. Interestingly, although these mutations are supposed to be largely derived from 8-oxoG, the direct sequence context of the mutations does not reflect the bias of a guanine 3′ of 8-oxoG [16]. It therefore has to be considered that not only the generation and repair of 8-oxoG may be affected by direct sequence context, but also the mutagenic properties, both for 8-oxoG-

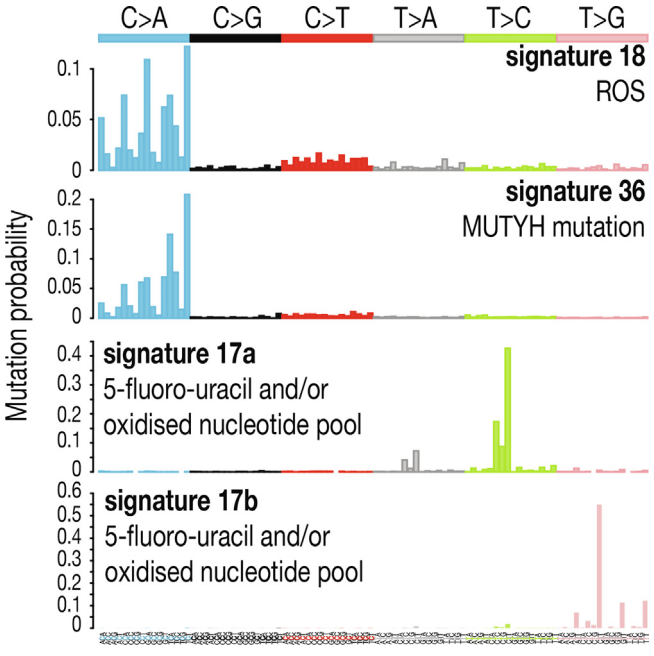

Fig. 6. Oxidative DNA damage dependent mutational signatures. Oxidative DNA damage has been associated with several mutational signatures, fingerprints of mutagenic processes in the genome that can be extracted using non-negative matrix factorization. First, Signature 18 is associated with ROS and dominated by C-to-A mutations with preceding and following adenine or thymine. Signature 36 gives a similar profile and has been linked to somatic MUTYH mutations. Signatures 17a and b have been connected both to oxidative DNA damage from an oxidized nucleotide pool and treatment with 5-fluoro-uracil. Mutations occur in a very specific trinucleotide context, most distinctly T-to-G in the context of TTG.

adenine mismatches and the A-rule of adenine incorporation opposite AP-sites. Alternatively, direct sequence context may be biased by the mechanisms leading to differential mutation distribution on a larger scale. Depletion of C-to-A mutations in promoters and coding sequence can explain an underrepresentation of preceding cytosines and guanines to some extent.

Signature 36 is also a signature linked to 8-oxoG. Similar to Signature 18 it is dominated by C-to-A mutations in similar sequence context and has been connected to biallelic germline or somatic *MUTYH* mutations [169]. It is particularly prevalent in endocrine pancreatic carcinoma and non-hodgkin B-cell lymphomas [159].

Signatures 17a and 17b show connection to oxidative DNA damage, although a precise etiology is not fully clear. They are frequent in stomach and esophagus adenocarcinoma, but also in other cancer types, such as non-hodgkin B-cell lymphoma. Signature 17a has characteristic T-to-C mutations, particularly in a CTT context. Signature 17b is characterized by T-to-G mutations most frequently observed also in a CTT context. One cause was found in treatment with 5-flouro-uracil [170,171], which can however not explain its "natural" occurrence. The latter is suspected to originate from incorporation of 8-oxoG nucleotides from the nucleotide pool [22]. Within its prevalence in esophagus adenocarcinoma, the signature can be connected to conditions that lead to increased acid reflux, i.e. alcohol consumption and the patient's body mass index (based on data from The Cancer Genome Atlas; unpublished).

With help of mutational signatures, it can be determined how oxidative DNA damage-derived mutations distribute in the genome. Not surprisingly, the four signatures are not associated with a transcriptional strand bias [159], a bias one would expect from mutagenesis mechanisms that are repaired through transcription-coupled repair. This is however different for the signatures' relation to the replication strand. Signature 18 and particularly Signature 17 are both associated with strand matching exposure and a positive slope for replication timing [22], which means that the later a given position in the genome is replicated, the higher the mutation rate. In general, higher mutation rates in late replicating DNA can be explained through the increased activity of error prone polymerases [172] and increased incorporation of ribonucleotides [173], which together leads to increased accumulation of mutations in heterochromatin in general [174,175], where late replicating DNA is typically located. Given the accumulation of oxidative DNA damage in heterochromatin, this bias in mutation rates for this mutagenic mechanism may also be explained through the underlying damage.

Differentiating the distinct levels of resolution (Fig. 7), accumulation of mutations from oxidative DNA damage in heterochromatin represents the distribution of mutations at chromatin domain resolution. At the resolution of genes and regulatory features, differences of mutation rates also reflect the patterns of oxidative DNA damage distribution [16,17] with reduced mutation rates in high GC content, which includes many major functionally important elements, such as promoters, enhancers, coding sequence, and anchors of chromatin architecture [17]. These mutation patterns show a striking resemblance to the patterns derived from mismatch repair [112,113]. At the same time, they reflect oxidative DNA damage distribution. Because polymerase proofreading and post-replicative mismatch repair would also be able to recognize 8-oxoG-adenine mismatches, it is not clear, how much each layer of regulation contributes to the final distribution of mutations. How much do the mechanisms described by Supek et al for mismatch repair shaping the somatic mutation rate across the human genome [114] also apply to mutations derived from oxidative DNA damage?

At a nucleosome scale, mutations from oxidative DNA damage reflect 8-oxoG levels, and accessibility seems to be the crucial determinant. Mutations derived from Signatures 17 and 18 in

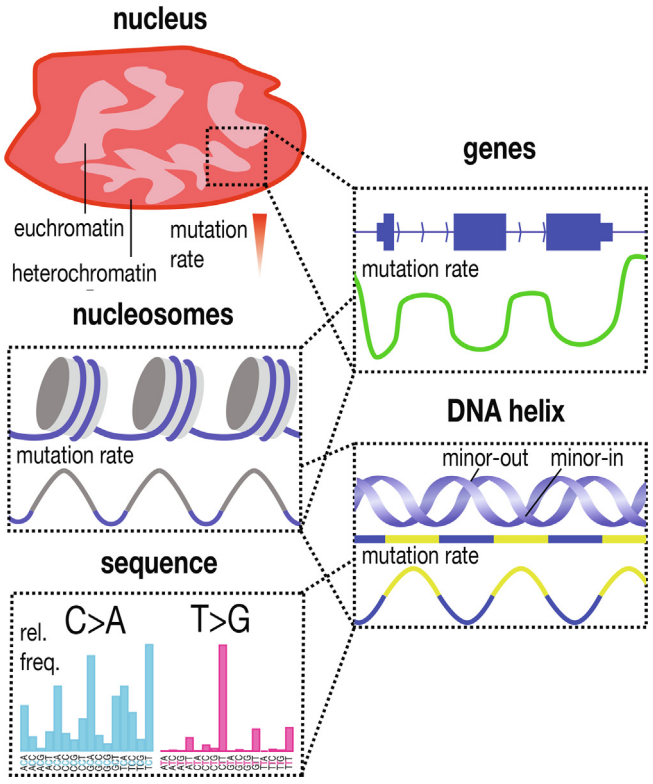

Fig. 7. Mutation rates from oxidative DNA damage show specificity at different levels of resolution from eu- and heterochromatin to the immediate sequence context. Mutation rates derived from oxidative DNA damage show distinct distributions at multiple levels of resolution. On the scale of chromatin domains, heterochromatin accumulates the major mutation load. Coding sequence of genes as well as GC rich regulatory sequences are depleted, while nucleosome positions in general are also enriched in mutations. At the level of DNA secondary structure, mutations are enriched at positions, where the minor groove of the helix faces the nucleosome. At the immediate sequence context, C-to-A mutations are enriched for preceding and following adenine or thymine. T-to-G mutations occur most frequently in a context of CTT.

breast cancer [176], and generally mutations in esophageal adenocarcinoma for example are enriched on the dyad of nucleosome positions [21], which could be interpreted as lower repair levels of mutations from misincorporated 8-oxoG and genomic 8-oxoG on regions covered by nucleosomes. Within the nucleosomes, mutations are enriched at sites of the minor groove facing the histone with strong evidence that the reason can be found in base excision repair being more efficient, when the minor groove faces the outside of the nucleosome. Although less strongly, higher mutation rate of Signature 18-dependent mutations were also found enriched in DNA with the minor groove facing the nucleosome. As *in vitro* studies suggest increased 8-oxoG generation in DNA with the minor groove facing away from the nucleosome [177], this distribution is likely due to the differences in base excision repair accessibility and efficiency [21].

At the finest level, i.e. the immediate sequence content, mutations from oxidative DNA damage are associated with distinct trinucleotide patterns. While this profile could indicate relevance of immediate sequence content for selectivity of the damage and repair processes regarding the direct sequence context for Signature 17b, the depletion of mutations in DNA of high GC content may explain the distinct profiles for Signatures 18 and 36, as the sequence content of the related mutations indicates preference for preceding and following adenine or thymine, while 8-oxoG is biased of a guanine 3′ of 8-oxoG [16].

In summary, the available data on the distribution of mutations leaves many open questions. We do understand that oxidative DNA

damage derived mutations distribute heterogeneously over the genome at several levels of resolution, and accessibility of DNA to base excision repair emerges as a main determinant of damage distribution on the nucleosome and chromatin domain levels. The precise mechanisms and their interaction that lead to the distribution at the level of genes and regulatory elements are largely still elusive. Mutations are generally following distributions similar to 8-oxoG, but it is largely unclear, how much of the damage distribution is due to the specificity in damage impact and how much of it is mediated by repair of the damage through BER. If repair is prioritizing, how is this regulated in the context of genome functionality and in different tissues? Also, en-route from the damage to the mutation, there are multiple layers that are not understood. What is the proportion of C-to-A mutations derived through mismatches as opposed to the application of the A-rule opposite an AP site? How much can be attributed through mismatches during replication as opposed to erroneous repair? This is of particular relevance for T-to-G mutations, where action of MUTYH on mis-incorporated 8-oxoG would induce mutations rather than reversing the damage. It can be expected that at each layer of resolution, a different combination of targeted mechanisms is contributing to the distribution, from the strong biases in regards to the preceding to following nucleotide, the helix orientation and secondary structures, distribution over gene elements, chromatin domains, eu- and hetero-chromatin/early and late replicating DNA.

Finally, targeted mechanisms that induce mutations via oxidative DNA damage may leave their mark in a region-specific manner, such as through the enzymatic function of LSD1.

Careful dissection of the mechanistic basis of mutation distribution does still require new developments in statistical methods, understanding of the underlying damage, as well as careful dissection of how all these processes act together, condition- and tissue-specifically.

This may open possibilities to advance the understanding of cancer evolution, the action of driver genes on mutation distribution, somatic mutagenesis, and even possibly manipulating the process.

10. Conclusions

The genomics of oxidative DNA damage, its repair and mutagenesis is a continuously growing field. This is made possible through biological method development to measure oxidative DNA damage genome-wide, development of data analysis methods, and methods to extract the genomic distribution of mutagenic mechanisms from mutation data. Each method and approach is characterized by unique benefits and pitfalls, so that comprehensive insight can only be established taking all such approaches together.

We have now achieved a descriptive understanding of the distribution of damage and mutation at several levels of resolution and in relation to some genome features and conditions. Mutation patterns are largely following the distribution of 8-oxoG at several layers of resolution. The distribution of the damage is to a large extent determined by accessibility of the DNA to base excision repair in combination with mechanisms of damage impact and most likely targeting of repair in the context of genome functionality.

We are however lacking detailed knowledge of the underlying mechanisms, the different relative contributions of the multiple levels of regulation and how these mechanisms act tissue-specifically. In addition, several mechanisms have been described for how oxidative DNA damage is also taking an active role in gene regulation and genome function. Such function has to be balanced with the risks of inducing genome instability and mutation. How does this balance affect diseases such as cancer and neurodegeneration?

Many DNA damage types may be caused simultaneously, e.g. strand breaks or pyrimidine dimers together with oxidative DNA damage. While we understand more about each separate DNA damage type and repair pathway, very little is known about the cross-talk of the repair pathways, especially when different damage occurs in close proximity, and the interaction with chromatin and genome function.

To understand these contexts, we need more specific mechanistic experiments, higher resolution and new statistical methods to address mutagenic mechanisms from mutation data. Tissue-specificity would be possible to address through even further resequencing of tumor samples and more healthy tissues to achieve the numbers needed to understand somatic mutagenesis, aided by single cell methods for oxidative DNA damage measurements and multi-omics data integration with functional genomics data. A better understanding of oxidative DNA damage distribution and improved methodology as well as new algorithms may also help to compensate for artifacts in genome sequencing data.

The genomics view on oxidative DNA damage, repair, and mutagenesis has opened an interdisciplinary field that combines chemical and biochemical expertise with genomics methods. It has revealed a large number of processes that regulate and balance how ROS damage the DNA region-specifically and how this is repaired in conjunction with gene expression regulation and emergence of mutations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

I would like to thank the whole field of oxidative DNA damage genomics for their contribution and would like to apologize that I could not cite all the papers that may be relevant. In addition, I am most grateful to Claudia Gebhard, Joanna Loizou, and Alexis Lomakin for careful reading of the manuscript.

References

[1] Markkanen E. Not breathing is not an option: How to deal with oxidative DNA damage. DNA Repair (Amst) 2017;59:82–105.
[2] Paiva CN, Bozza MT. Are reactive oxygen species always detrimental to pathogens. Antioxid Redox Signal 2014;20:1000–37.
[3] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked. Free Radic Biol Med 2010;49:1603–16.
[4] van Loon B, Markkanen E, Hübscher U. Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. DNA Repair (Amst) 2010;9:604–16.
[5] Roots R, Okada S. Protection of DNA molecules of cultured mammalian cells from radiation-induced single-strand scissions by various alcohols and SH compounds. Int J Radiat Biol Relat Stud Phys Chem Med 1972;21:329–42.
[6] Ward JF. Complexity of damage produced by ionizing radiation. Cold Spring Harb Symp Quant Biol 2000;65:377–82.
[7] Lehner S. Biomolecular action of ionizing radiation. CRC Press; 2007.
[8] Sasani TA, Pedersen BS, Gao Z, Baird L, Przeworski M, Jorde LB, et al. Large, three-generation human families reveal post-zygotic mosaicism and variability in germline mutation accumulation. Elife 2019;8.
[9] Crow JF. The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet 2000.
[10] Tubbs A, Nussenzweig A. Endogenous DNA damage as a source of genomic instability in cancer. Cell 2017;168:644–56.
[11] Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. Science 2015;349:1483–9.
[12] Finkel T, Serrano M, Blasco MA. The common biology of cancer and ageing. Nature 2007;448:767–74.

[13] Kennedy SR, Loeb LA, Herr AJ. Somatic mutations in aging, cancer and neurodegeneration. Mech Ageing Dev 2012;133:118–26.
[14] Lodato MA, Rodin RE, Bohrson CL, Coulter ME, Barton AR, Kwon M, et al. Aging and neurodegeneration are associated with increased mutations in single human neurons. Science 2018;359:555–9.
[15] Ding Y, Fleming AM, Burrows CJ. Sequencing the mouse genome for the oxidatively modified base 8-oxo-7,8-dihydroguanine by OG-seq. J Am Chem Soc 2017;139:2569–72.
[16] Wu J, Mckeague M, Sturla SJ. Nucleotide-resolution genome-wide mapping of oxidative DNA damage by click-code-seq. J Am Chem Soc 2018.
[17] Poetsch AR, Boulton SJ, Luscombe NM. Genomic landscape of oxidative DNA damage and repair reveals regioselective protection from mutagenesis. Genome Biol 2018;19:215.
[18] Amente S, Di Palo G, Scala G, Castrignanò T, Gorini F, Cocozza S, et al. Genome-wide mapping of 8-oxo-7,8-dihydro-2′-deoxyguanosine reveals accumulation of oxidatively-generated damage at DNA replication origins within transcribed long genes of mammalian cells. Nucleic Acids Res 2019;47:221–36.
[19] Pastukh V, Ruchko M, Gorodnya O, Wilson GL, Gillespie MN. Sequence-specific oxidative base modifications in hypoxia-inducible genes. Free Radic Biol Med 2007;43:1616–26.
[20] Park JW, Han YI, Kim TM, Yeom SC, Kang J, Park J. 8-OxoG in GC-rich Sp1 binding sites enhances gene transcription during adipose tissue development in juvenile mice. Sci Rep 2019;9:15618.
[21] Pich O, Muiños F, Sabarinathan R, Reyes-Salazar I, Gonzalez-Perez A, Lopez-Bigas N. Somatic and germline mutation periodicity follow the orientation of the DNA minor groove around nucleosomes. Cell 2018;175:1074–1087.e18.
[22] Tomkova M, Tomek J, Kriaucionis S, Schuster-Böckler B. Mutational signature distribution varies with DNA replication timing and strand asymmetry. Genome Biol 2018;19:129.
[23] Moore SP, Toomire KJ, Strauss PR. DNA modifications repaired by base excision repair are epigenetic. DNA Repair (Amst) 2013;12:1152–8.
[24] Park J, Park JW, Oh H, Maria FS, Kang J, Tian X. Gene-specific assessment of guanine oxidation as an epigenetic modulator for cardiac specification of mouse embryonic stem cells. PLoS One 2016;11:e0155792.
[25] Fleming AM, Burrows CJ. 8-Oxo-7,8-dihydroguanine, friend and foe: Epigenetic-like regulator versus initiator of mutagenesis. DNA Repair (Amst) 2017;56:75–83.
[26] Coluzzi E, Colamartino M, Cozzi R, Leone S, Meneghini C, O'Callaghan N, et al. Oxidative stress induces persistent telomeric DNA damage responsible for nuclear morphology change in mammalian cells. PLoS One 2014;9:e110963.
[27] Fouquerel E, Barnes RP, Uttam S, Watkins SC, Bruchez MP, Opresko PL. Targeted and persistent 8-oxoguanine base damage at telomeres promotes telomere loss and crisis. Mol Cell 2019;75:117–130.e6.
[28] von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002;27:339–44.
[29] Allgayer J, Kitsera N, Bartelt S, Epe B, Khobta A. Widespread transcriptional gene inactivation initiated by a repair intermediate of 8-oxoguanine. Nucleic Acids Res 2016;44:7267–80.
[30] Tornaletti S, Maeda LS, Kolodner RD, Hanawalt PC. Effect of 8-oxoguanine on transcription elongation by T7 RNA polymerase and mammalian RNA polymerase II. DNA Repair (Amst) 2004;3:483–94.
[31] Liu ZJ, Martínez Cuesta S, van Delft P, Balasubramanian S. Sequencing abasic sites in DNA at single-nucleotide resolution. Nat Chem 2019;11:629–37.
[32] The International Cancer Genome Consortium et al. International network of cancer genome projects. Nature 2010;464:993–8.
[33] The Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 2013;45:1113–20.
[34] Blokzijl F et al. Tissue-specific mutation accumulation in human adult stem cells during life. Nature 2016;538:260–4.
[35] Lee-Six H, Øbro NF, Shepherd MS, Grossmann S, Dawson K, Belmonte M, et al. Population dynamics of normal human blood inferred from somatic mutations. Nature 2018;561:473–8.
[36] Martincorena I, Seshasayee AS, Luscombe NM. Evidence of non-random mutation rates suggests an evolutionary risk management strategy. Nature 2012;485:95–8.
[37] Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, et al. Somatic mutant clones colonize the human esophagus with age. Science 2018;362:911–7.
[38] Cadet J, Douki T, Ravanat JL. One-electron oxidation of DNA and inflammation processes. Nat Chem Biol 2006;2:348–9.
[39] Margolin Y, Cloutier JF, Shafirovich V, Geacintov NE, Dedon PC. Paradoxical hotspots for guanine oxidation by a chemical mediator of inflammation. Nat Chem Biol 2006;2:365–6.
[40] Cadet J, Davies KJA, Medeiros MH, Di Mascio P, Wagner JR. Formation and repair of oxidatively generated damage in cellular DNA. Free Radic Biol Med 2017;107:13–34.
[41] Di Mascio P, Martinez GR, Miyamoto S, Ronsein GE, Medeiros MHG, Cadet J. Singlet molecular oxygen reactions with nucleic acids, lipids, and proteins. Chem Rev 2019;119:2043–86.
[42] Lindahl T. Instability and decay of the primary structure of DNA. Nature 1993;362:709–15.
[43] Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature 1992;355:273–5.
[44] Inoue M, Kamiya H, Fujikawa K, Ootsuyama Y, Murata-Kamiya N, Osaki T, Yasumoto K, Kasai H. Induction of chromosomal gene mutations in Escherichia coli by direct incorporation of oxidatively damaged nucleotides. New evaluation method for mutagenesis by damaged DNA precursors in vivo. J. Biol. Chem. 1998;273(18):11069–74. https://doi.org/10.1074/jbc.273.18.11069.
[45] Satou K, Kawai K, Kasai H, Harashima H, Kamiya H. Mutagenic effects of 8-hydroxy-dGTP in live mammalian cells. Free Radic Biol Med 2007;42:1552–60.
[46] Satou K, Hori M, Kawai K, Kasai H, Harashima H, Kamiya H. Involvement of specialized DNA polymerases in mutagenesis by 8-hydroxy-dGTP in human cells. DNA Repair (Amst) 2009;8:637–42.
[47] David SS, O'Shea VL, Kundu S. Base-excision repair of oxidative DNA damage. Nature 2007;447:941–50.
[48] Hamilton ML, Guo Z, Fuller CD, Van Remmen H, Ward WF, Austad SN, et al. A reliable assessment of 8-oxo-2-deoxyguanosine levels in nuclear and mitochondrial DNA using the sodium iodide method to isolate DNA. Nucleic Acids Res 2001;29:2117–26.
[49] Lindahl T, Nyberg B. Rate of depurination of native deoxyribonucleic acid. Biochemistry 1972;11:3610–8.
[50] Yamaguchi R, Hirano T, Asami S, Chung MH, Sugita A, Kasai H. Increased 8-hydroxyguanine levels in DNA and its repair activity in rat kidney after administration of a renal carcinogen, ferric nitrilotriacetate. Carcinogenesis 1996;17:2419–22.
[51] Gedik CM, Collins A, ESCODD. Establishing the background level of base oxidation in human lymphocyte DNA. FASEB J 2005;19:82–4.
[52] Swenberg JA, Lu K, Moeller BC, Gao L, Upton PB, Nakamura J, et al. Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology, and risk assessment. Toxicol Sci 2011;120(Suppl 1): S130–45.
[53] Rahimoff R, Kosmatchev O, Kirchner A, Pfaffeneder T, Spada F, Brantl V, et al. 5-Formyl- and 5-carboxydeoxycytidines do not cause accumulation of harmful repair intermediates in stem cells. J Am Chem Soc 2017;139:10359–64.
[54] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012;5:9–19.
[55] Ramon O, Sauvaigo S, Gasparutto D, Faure P, Favier A, Cadet J. Effects of 8-oxo-7,8-dihydro-2′-deoxyguanosine on the binding of the transcription factor Sp1 to its cognate target DNA sequence (GC box). Free Radic Res 1999;31:217–29.
[56] Hailer-Morrison MK, Kotler JM, Martin BD, Sugden KD. Oxidized guanine lesions as modulators of gene transcription. Altered p50 binding affinity and repair shielding by 7,8-dihydro-8-oxo-2′-deoxyguanosine lesions in the NF-kappaB promoter element. Biochemistry 2003;42:9761–70.
[57] Khobta A, Anderhub S, Kitsera N, Epe B. Gene silencing induced by oxidative DNA base damage: association with local decrease of histone H4 acetylation in the promoter region. Nucleic Acids Res 2010;38:4285–95.
[58] Larsen E, Kwon K, Coin F, Egly JM, Klungland A. Transcription activities at 8-oxoG lesions in DNA. DNA Repair (Amst) 2004;3:1457–68.
[59] Ruchko MV, Gorodnya OM, Pastukh VM, Swiger BM, Middleton NS, Wilson GL, et al. Hypoxia-induced oxidative base modifications in the VEGF hypoxia-response element are associated with transcriptionally active nucleosomes. Free Radic Biol Med 2009;46:352–9.
[60] Pastukh V, Roberts JT, Clark DW, Bardwell GC, Patel M, Al-Mehdi AB, et al. An oxidative DNA "damage" and repair mechanism localized in the VEGF promoter is important for hypoxia-induced VEGF mRNA expression. Am J Physiol Lung Cell Mol Physiol 2015;309:L1367–75.
[61] Fleming AM, Ding Y, Burrows CJ. Oxidative DNA damage is epigenetic by regulating gene transcription via base excision repair. Proc Natl Acad Sci U S A 2017;114:2604–9.
[62] Wang R, Hao W, Pan L, Boldogh I, Ba X. The roles of base excision repair enzyme OGG1 in gene expression. Cell Mol Life Sci 2018;75:3741–50.
[63] Fleming AM, Zhu J, Ding Y, Burrows CJ. 8-Oxo-7,8-dihydroguanine in the context of a gene promoter g-quadruplex is an on-off switch for transcription. ACS Chem Biol 2017;12:2417–26.
[64] Kwok CK, Merrick CJ. G-Quadruplexes: prediction, characterization, and biological application. Trends Biotechnol 2017.
[65] Graham MK, Meeker A. Telomeres and telomerase in prostate cancer development and therapy. Nat Rev Urol 2017;14:607–19.
[66] Sedletska Y, Radicella JP, Sage E. Replication fork collapse is a major cause of the high mutation frequency at three-base lesion clusters. Nucleic Acids Res 2013;41:9339–48.
[67] Boiteux S, Guillet M. Abasic sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. DNA Repair (Amst) 2004;3:1–12.
[68] Sczepanski JT, Wong RS, McKnight JN, Bowman GD, Greenberg MM. Rapid DNA–protein cross-linking and strand scission by an abasic site in a nucleosome core particle. Proc Natl Acad Sci 2010;107:22475–80.
[69] Bravard A, Vacher M, Gouget B, Coutant A, de Boisferon FH, Marsin S, et al. Redox regulation of human OGG1 activity in response to cellular oxidative stress. Mol Cell Biol 2006;26:7430–6.
[70] Morreall J, Limpose K, Sheppard C, Kow YW, Werner E, Doetsch PW. Inactivation of a common OGG1 variant by TNF-alpha in mammalian cells. DNA Repair (Amst) 2015;26:15–22.
[71] Yoshihara M, Jiang L, Akatsuka S, Suyama M, Toyokuni S. Genome-wide profiling of 8-oxoguanine reveals its association with spatial positioning in nucleus. DNA Res 2014;21:603–12.

[72] Poetsch A.R. AP-seq, a method to measure apurinic sites and small base adducts genome-wide. Methods in Molecular Biology - The Nucleus, 3rd edition (Springer) 2020.
[73] Kubo K, Ide H, Wallace SS, Kow YW. A novel, sensitive, and specific assay for abasic sites, the most commonly produced DNA lesion. Biochemistry 1992;31:3703–8.
[74] Atamna H, Cheung I, Ames BN. A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. Proc Natl Acad Sci USA 2000;97:686–91.
[75] Nakamura J, Walker VE, Upton PB, Chiang SY, Kow YW, Swenberg JA. Highly sensitive apurinic/apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. Cancer Res 1998;58:222–5.
[76] Nakano T, Terato H, Yoshioka Y, Ohyama Y, Kubo K, Ide H. Detection of NO-induced DNA lesions by the modified aldehyde reactive probe (ARP) assay. Nucleic Acids Res Suppl 2002;239–240.
[77] Roberts KP, Sobrino JA, Payton J, Mason LB, Turesky RJ. Determination of apurinic/apyrimidinic lesions in DNA with high-performance liquid chromatography and tandem mass spectrometry. Chem Res Toxicol 2006;19:300–9.
[78] Mitchell DL, Meador J, Paniker L, Gasparutto D, Jeffrey WH, Cadet J. Development and application of a novel immunoassay for measuring oxidative DNA damage in the environment. Photochem Photobiol 2002;75:257–65.
[79] Serrano J, Palmeira CM, Wallace KB, Kuehl DW. Determination of 8-hydroxydeoxyguanosine in biological tissue by liquid chromatography/electrospray ionization-mass spectrometry/mass spectrometry. Rapid Commun Mass Spectrom 1996;10:1789–91.
[80] Krishnamurthy N, Haraguchi K, Greenberg MM, David SS. Efficient removal of formamidopyrimidines by 8-oxoguanine glycosylases. Biochemistry 2008;47:1043–50.
[81] David SS, Williams SD. Chemistry of glycosylases and endonucleases involved in base-excision repair. Chem Rev 1998;98:1221–62.
[82] Raiber EA, Beraldi D, Ficz G, Burgess HE, Branco MR, Murat P, et al. Genome-wide distribution of 5-formylcytosine in embryonic stem cells is associated with transcription and depends on thymine DNA glycosylase. Genome Biol 2012;13:R69.
[83] Ravanat JL, Douki T, Duez P, Gremaud E, Herbert K, Hofer T, et al. Cellular background level of 8-oxo-7,8-dihydro-2′-deoxyguanosine: an isotope based method to evaluate artefactual oxidation of DNA during its extraction and subsequent work-up. Carcinogenesis 2002;23:1911–8.
[84] Costello M, Pugh TJ, Fennell TJ, Stewart C, Lichtenstein L, Meldrim JC, et al. Discovery and characterization of artifactual mutations in deep coverage targeted capture sequencing data due to oxidative DNA damage during sample preparation. Nucleic Acids Res 2013;41:e67.
[85] Akatsuka S et al. Contrasting genome-wide distribution of 8-hydroxyguanine and acrolein-modified adenine during oxidative stress-induced renal carcinogenesis. Am J Pathol 2006;169:1328–42.
[86] van Steensel B, Belmont AS. Lamina-associated domains: links with chromosome architecture, heterochromatin, and gene repression. Cell 2017;169:780–91.
[87] Larson AG, Elnatan D, Keenen MM, Trnka MJ, Johnston JB, Burlingame AL, et al. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. Nature 2017;547:236–40.
[88] Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, Karpen GH. Phase separation drives heterochromatin domain formation. Nature 2017;547:241–5.
[89] Campalans A, Amouroux R, Bravard A, Epe B, Radicella JP. UVA irradiation induces relocalisation of the DNA repair protein hOGG1 to nuclear speckles. J Cell Sci 2007;120:23–32.
[90] Amouroux R, Campalans A, Epe B, Radicella JP. Oxidative stress triggers the preferential assembly of base excision repair complexes on open chromatin regions. Nucleic Acids Res 2010;38:2878–90.
[91] Kubota Y, Takanami T, Higashitani A, Horiuchi S. Localization of X-ray cross complementing gene 1 protein in the nuclear matrix is controlled by casein kinase II-dependent phosphorylation in response to oxidative damage. DNA Repair (Amst) 2009;8:953–60.
[92] Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Löbrich M, et al. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Mol Cell 2008;31:167–77.
[93] Kanu N, Penicud K, Hristova M, Wong B, Irvine E, Plattner F, et al. The ATM cofactor ATMIN protects against oxidative stress and accumulation of DNA damage in the aging brain. J Biol Chem 2010;285:38534–42.
[94] Blake SM, Stricker SH, Halavach H, Poetsch AR, Cresswell G, Kelly G, et al. Inactivation of the ATMIN/ATM pathway protects against glioblastoma formation. Elife 2016;5.
[95] Beard BC, Wilson SH, Smerdon MJ. Suppressed catalytic activity of base excision repair enzymes on rotationally positioned uracil in nucleosomes. Proc Natl Acad Sci USA 2003;100:7465–70.
[96] Menoni H, Gasparutto D, Hamiche A, Cadet J, Dimitrov S, Bouvet P, et al. ATP-dependent chromatin remodeling is required for base excision repair in conventional but not in variant H2A.Bbd nucleosomes. Mol Cell Biol 2007;27:5949–56.
[97] Menoni H, Di Mascio P, Cadet J, Dimitrov S, Angelov D. Chromatin associated mechanisms in base excision repair – nucleosome remodeling and DNA transcription, two key players. Free Radic Biol Med 2017;107:159–69.
[98] Nilsen H, Lindahl T, Verreault A. DNA base excision repair of uracil residues in reconstituted nucleosome core particles. EMBO J 2002;21:5943–52.
[99] Bhakat KK, Mokkapati SK, Boldogh I, Hazra TK, Mitra S. Acetylation of human 8-oxoguanine-DNA glycosylase by p300 and its role in 8-oxoguanine repair in vivo. Mol Cell Biol 2006;26:1654–65.
[100] Margolin Y, Shafirovich V, Geacintov NE, DeMott MS, Dedon PC. DNA sequence context as a determinant of the quantity and chemistry of guanine oxidation produced by hydroxyl radicals and one-electron oxidants. J Biol Chem 2008;283:35569–78.
[101] Senthilkumar K, Grozema FC, Guerra CF, Bickelhaupt FM, Siebbeles LD. Mapping the sites for selective oxidation of guanines in DNA. J Am Chem Soc 2003;125:13658–9.
[102] Rhee DB, Ghosh A, Lu J, Bohr VA, Liu Y. Factors that influence telomeric oxidative base damage and repair by DNA glycosylase OGG1. DNA Repair (Amst) 2011;10:34–44.
[103] Zhou J, Liu M, Fleming AM, Burrows CJ, Wallace SS. Neil3 and NEIL1 DNA glycosylases remove oxidative damages from quadruplex DNA and exhibit preferences for lesions in the telomeric sequence context. J Biol Chem 2013;288:27263–72.
[104] Cogoi S, Ferino A, Miglietta G, Pedersen EB, Xodo LE. The regulatory G4 motif of the Kirsten ras (KRAS) gene is sensitive to guanine oxidation: implications on transcription. Nucleic Acids Res 2018;46:661–76.
[105] Broxson C, Hayner JN, Beckett J, Bloom LB, Tornaletti S. Human AP endonuclease inefficiently removes abasic sites within G4 structures compared to duplex DNA. Nucleic Acids Res 2014;42:7708–19.
[106] Georgakopoulos-Soares I, Morganella S, Jain N, Hemberg M, Nik-Zainal S. Noncanonical secondary structures arising from non-B DNA motifs are determinants of mutagenesis. Genome Res 2018;28:1264–71.
[107] Farkash EA, Kao GD, Horman SR, Prak ET. Gamma radiation increases endonuclease-dependent L1 retrotransposition in a cultured cell assay. Nucleic Acids Res 2006;34:1196–204.
[108] Liu N, Lee CH, Swigut T, Grow E, Gu B, Bassik MC, et al. Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. Nature 2018;553:228–32.
[109] Fleming AM, Zhu J, Ding Y, Burrows CJ. Location dependence of the transcriptional response of a potential G-quadruplex in gene promoters under oxidative stress. Nucleic Acids Res 2019;47:5049–60.
[110] Weiner A, Hsieh TH, Appleboim A, Chen HV, Rahat A, Amit I, et al. High-resolution chromatin dynamics during a yeast stress response. Mol Cell 2015;58:371–86.
[111] Li F, Mao G, Tong D, Huang J, Gu L, Yang W, et al. The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutSα. Cell 2013;153:590–600.
[112] Frigola J, Sabarinathan R, Mularoni L, Muiños F, Gonzalez-Perez A, López-Bigas N. Reduced mutation rate in exons due to differential mismatch repair. Nat Genet 2017;49:1684–92.
[113] Supek F, Lehner B. Differential DNA mismatch repair underlies mutation rate variation across the human genome. Nature 2015;521:81–4.
[114] Supek F, Lehner B. Scales and mechanisms of somatic mutation rate variation across the human genome. DNA Repair (Amst) 2019;102647.
[115] Mao P, Brown AJ, Malc EP, Mieczkowski PA, Smerdon MJ, Roberts SA, et al. Genome-wide maps of alkylation damage, repair, and mutagenesis in yeast reveal mechanisms of mutational heterogeneity. Genome Res 2017;27:1674–84.
[116] Natale F, Rapp A, Yu W, Maiser A, Harz H, Scholl A, et al. Identification of the elementary structural units of the DNA damage response. Nat Commun 2017;8:15760.
[117] Kim N, Jinks-Robertson S. Transcription as a source of genome instability. Nat Rev Genet 2012;13:204–14.
[118] Sondka Z, Bamford S, Cole CG, Ward SA, Dunham I, Forbes SA. The COSMIC Cancer Gene Census: describing genetic dysfunction across all human cancers. Nat Rev Cancer 2018;18:696–705.
[119] Jiang D, Malla S, Fu YJ, Choudhary D, Rusling JF. Direct LC-MS/MS detection of guanine oxidations in exon 7 of the p53 tumor suppressor gene. Anal Chem 2017;89:12872–9.
[120] Kawai K, Wata Y, Hara M, Tojo S, Majima T. Regulation of one-electron oxidation rate of guanine by base pairing with cytosine derivatives. J Am Chem Soc 2002;124:3586–90.
[121] Ming X, Matter B, Song M, Veliath E, Shanley R, Jones R, et al. Mapping structurally defined guanine oxidation products along DNA duplexes: influence of local sequence context and endogenous cytosine methylation. J Am Chem Soc 2014;136:4223–35.
[122] Sassa A, Çağlayan M, Dyrkheeva NS, Beard WA, Wilson SH. Base excision repair of tandem modifications in a methylated CpG dinucleotide. J Biol Chem 2014;289:13996–4008.
[123] Kasymov RD, Grin IR, Endutkin AV, Smirnov SL, Ishchenko AA, Saparbaev MK, et al. Excision of 8-oxoguanine from methylated CpG dinucleotides by human 8-oxoguanine DNA glycosylase. FEBS Lett 2013;587:3129–34.
[124] Xia L, Huang W, Bellani M, Seidman MM, Wu K, Fan D, Nie Y, Cai Y, Zhang YW, Yu LR, Li H, Zahnow CA, Xie W, Chiu Yen RW, Rassool FV, Baylin SB. CHD4 has oncogenic functions in initiating and maintaining epigenetic suppression of multiple tumor suppressor genes. Cancer Cell 2017;31(5):653–668.e7. https://doi.org/10.1016/j.ccell.2017.04.005.
[125] Kamenisch Y, Fousteri M, Knoch J, von Thaler AK, Fehrenbacher B, Kato H, et al. Proteins of nucleotide and base excision repair pathways interact in

mitochondria to protect from loss of subcutaneous fat, a hallmark of aging. J Exp Med 2010;207:379–90.
[126] Noren Hooten N, Kompaniez K, Barnes J, Lohani A, Evans MK. Poly(ADP-ribose) polymerase 1 (PARP-1) binds to 8-oxoguanine-DNA glycosylase (OGG1). J Biol Chem 2011;286:44679–90.
[127] Pal R, Ramdzan ZM, Kaur S, Duquette PM, Marcotte R, Leduy L, et al. CUX2 protein functions as an accessory factor in the repair of oxidative DNA damage. J Biol Chem 2015;290:22520–31.
[128] Ramdzan ZM, Vadnais C, Pal R, Vandal G, Cadieux C, Leduy L, et al. RAS transformation requires CUX1-dependent repair of oxidative DNA damage. PLoS Biol 2014;12:e1001807.
[129] Ewing RM et al. Large-scale mapping of human protein-protein interactions by mass spectrometry. Mol Syst Biol 2007;3:89.
[130] Bhakat KK, Izumi T, Yang SH, Hazra TK, Mitra S. Role of acetylated human AP-endonuclease (APE1/Ref-1) in regulation of the parathyroid hormone gene. EMBO J 2003;22:6299–309.
[131] Yamamori T, DeRicco J, Naqvi A, Hoffman TA, Mattagajasingh I, Kasuno K, et al. SIRT1 deacetylates APE1 and regulates cellular base excision repair. Nucleic Acids Res 2010;38:832–45.
[132] Vascotto C, Fantini D, Romanello M, Cesaratto L, Deganuto M, Leonardi A, et al. APE1/Ref-1 interacts with NPM1 within nucleoli and plays a role in the rRNA quality control process. Mol Cell Biol 2009;29:1834–54.
[133] Kuninger DT, Izumi T, Papaconstantinou J, Mitra S. Human AP-endonuclease 1 and hnRNP-L interact with a nCaRE-like repressor element in the AP-endonuclease 1 promoter. Nucleic Acids Res 2002;30:823–9.
[134] Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. Proc Natl Acad Sci USA 1997;94:3633–8.
[135] Berquist BR, McNeill DR, Wilson DM. Characterization of abasic endonuclease activity of human Ape1 on alternative substrates, as well as effects of ATP and sequence context on AP site incision. J Mol Biol 2008;379:17–27.
[136] Fleming AM, Zhu J, Ding Y, Esders S, Burrows CJ. Oxidative modification of guanine in a potential Z-DNA-forming sequence of a gene promoter impacts gene expression. Chem Res Toxicol 2019;32:899–909.
[137] Cogoi S, Shchekotikhin AE, Xodo LE. HRAS is silenced by two neighboring G-quadruplexes and activated by MAZ, a zinc-finger transcription factor with DNA unfolding property. Nucleic Acids Res 2014;42:8379–88.
[138] Yin Y, Morgunova E, Jolma A, Kaasinen E, Sahu B, Khund-Sayeed S, et al. Impact of cytosine methylation on DNA binding specificities of human transcription factors. Science 2017;356.
[139] Tomkova M, Schuster-Böckler B. DNA modifications: naturally more error prone. Trends Genet 2018;34:627–38.
[140] Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, et al. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science 2008;319:202–6.
[141] Duncan BK, Miller JH. Mutagenic deamination of cytosine residues in DNA. Nature 1980;287:560–1.
[142] Cooper DN, Krawczak M. Cytosine methylation and the fate of CpG dinucleotides in vertebrate genomes. Hum Genet 1989;83:181–8.
[143] Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 1991;349:431–4.
[144] Markkanen E, Dorn J, Hübscher U. MUTYH DNA glycosylase: the rationale for removing undamaged bases from the DNA. Front Genet 2013;4:18.
[145] Loeb LA. Apurinic sites as mutagenic intermediates. Cell 1985;40:483–4.
[146] Sagher D, Strauss B. Insertion of nucleotides opposite apurinic/apyrimidinic sites in deoxyribonucleic acid during in vitro synthesis: uniqueness of adenine nucleotides. Biochemistry 1983;22:4518–26.
[147] Shibutani S, Takeshita M, Grollman AP. Translesional synthesis on DNA templates containing a single abasic site. A mechanistic study of the "A rule". J Biol Chem 1997;272:13916–22.
[148] Suzuki T, Kamiya H. Mutations induced by 8-hydroxyguanine (8-oxo-7,8-dihydroguanine), a representative oxidized base, in mammalian cells. Genes Environ 2017;39:2.
[149] Murugaesu N, Wilson GA, Birkbak NJ, Watkins T, McGranahan N, Kumar S, et al. Tracking the genomic evolution of esophageal adenocarcinoma through neoadjuvant chemotherapy. Cancer Discov 2015;5:821–31.
[150] Ross-Innes CS, Becq J, Warren A, Cheetham RK, Northen H, O'Donovan M, et al. Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. Nat Genet 2015;47:1038–46.
[151] Dulak AM et al. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. Nat Genet 2013;45:478–86.
[152] Nones K et al. Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis. Nat Commun 2014;5:5224.
[153] Gonzalez-Perez A, Sabarinathan R, Lopez-Bigas N. Local determinants of the mutational landscape of the human genome. Cell 2019;177:101–14.
[154] Kucab JE, Zou X, Morganella S, Joel M, Nanda AS, Nagy E, et al. A Compendium of Mutational Signatures of Environmental Agents. Cell 2019;177:821–836.e16.
[155] Arbeithuber B, Makova KD, Tiemann-Boege I. Artifactual mutations resulting from DNA lesions limit detection levels in ultrasensitive sequencing applications. DNA Res 2016;23:547–59.
[156] Ma X, Shao Y, Tian L, Flasch DA, Mulder HL, Edmonson MN, et al. Analysis of error profiles in deep next-generation sequencing data. Genome Biol 2019;20:50.
[157] Alexandrov LB et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
[158] Nik-Zainal S et al. Mutational processes molding the genomes of 21 breast cancers. Cell 2012;149:979–93.
[159] Alexandrov L, Kim J, Haradhvala NJ, Huang MN, Ng AWT, Boot A, Covington KR, Gordenin DA, Bergstrom E, Lopez-Bigas N, Klimczak LJ, McPherson JR, Morganella S, Sabarinathan R, Wheeler DA, Mustonen V, Getz G, Rozen SG, Stratton MR. The repertoire of mutational signatures in human cancer. bioRxiv 2018;322859.
[160] Connor F, Rayner TF, Aitken SJ, Feig C, Lukk M, Santoyo-Lopez J, et al. Mutational landscape of a chemically-induced mouse model of liver cancer. J Hepatol 2018;69:840–50.
[161] Meier B, Cooke SL, Weiss J, Bailly AP, Alexandrov LB, Marshall J, et al. elegans whole-genome sequencing reveals mutational signatures related to carcinogens and DNA repair deficiency. Genome Res 2014;24:1624–36.
[162] Zou X, Owusu M, Harris R, Jackson SP, Loizou JI, Nik-Zainal S. Validating the concept of mutational signatures with isogenic cell models. Nat Commun 2018;9:1744.
[163] Helleday T, Eshtad S, Nik-Zainal S. Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet 2014;15:585–98.
[164] Volkova NV, Meier B, Gonzalez-Huici V, Bertolini S, Gonzalez S, Abascal F, et al. Mutational signatures are jointly shaped by DNA damage and repair. bioRxiv 2019.
[165] Peña-Diaz J, Bregenhorn S, Ghodgaonkar M, Follonier C, Artola-Borán M, Castor D, et al. Noncanonical mismatch repair as a source of genomic instability in human cells. Mol Cell 2017;67:162.
[166] Zlatanou A, Despras E, Braz-Petta T, Boubakour-Azzouz I, Pouvelle C, Stewart GS, et al. The hMsh2-hMsh6 complex acts in concert with monoubiquitinated PCNA and Pol η in response to oxidative DNA damage in human cells. Mol Cell 2011;43:649–62.
[167] Petljak M et al. Characterizing mutational signatures in human cancer cell lines reveals episodic APOBEC mutagenesis. Cell 2019;176:1282–1294.e20.
[168] Supek F, Lehner B. Clustered mutation signatures reveal that error-prone DNA repair targets mutations to active genes. Cell 2017;170:534–547.e23.
[169] Rashid M, Fischer A, Wilson CH, Tiffen J, Rust AG, Stevens P, et al. Adenoma development in familial adenomatous polyposis and MUTYH-associated polyposis: somatic landscape and driver genes. J Pathol 2016;238:98–108.
[170] Pich O, Muiños F, Lolkema MP, Steeghs N, Gonzalez-Perez A, Lopez-Bigas N. The mutational footprints of cancer therapies. Nat Genet 2019;51:1732–40.
[171] Christensen S, Van der Roest B, Besselink N, Janssen R, Boymans S, Martens JWM, et al. 5-Fluorouracil treatment induces characteristic T>G mutations in human cancer. Nat Commun 2019;10:4571.
[172] Lujan SA, Clausen AR, Clark AB, MacAlpine HK, MacAlpine DM, Malc EP, et al. Heterogeneous polymerase fidelity and mismatch repair bias genome variation and composition. Genome Res 2014;24:1751–64.
[173] Reijns MAM, Kemp H, Ding J, de Procé SM, Jackson AP, Taylor MS. Lagging-strand replication shapes the mutational landscape of the genome. Nature 2015;518:502–6.
[174] Schuster-Böckler B, Lehner B. Chromatin organization is a major influence on regional mutation rates in human cancer cells. Nature 2012;488:504–7.
[175] Polak P, Lawrence MS, Haugen E, Stoletzki N, Stojanov P, Thurman RE, et al. Reduced local mutation density in regulatory DNA of cancer genomes is linked to DNA repair. Nat Biotechnol 2014;32:71–5.
[176] Morganella S et al. The topography of mutational processes in breast cancer genomes. Nat Commun 2016;7:11383.
[177] Liu Y, Liu Z, Geacintov NE, Shafirovich V. Proton-coupled hole hopping in nucleosomal and free DNA initiated by site-specific hole injection. Phys Chem Chem Phys 2012;14:7400–10.