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Surviving an Oxygen Atmosphere: DNA Damage and Repair

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Abstract

As a consequence of life's coexistence with the reactive diradical O_2 , cells have adapted biochemical defense mechanisms for protection from oxidative damage. Nevertheless, it is estimated that each cell's genomic DNA undergoes thousands of oxidative hits per day, and even more under conditions of stress. Unrepaired oxidative damage to DNA leads to mutations that underlie cancer, aging and neurological disease. Recent studies have helped unravel the oxidation chemistry of the DNA bases, and the myriad biochemical responses of DNA processing enzymes that battle against mutation. On the positive side, oxidative damage to nucleobases may accelerate the evolution of genomes and could have played a role in the ancestry of redox-active nucleoside cofactors as well as the adaptation of early life to changes in the environment.

When cyanobacteria gained the function of converting H_2O to O_2 during the Great Oxygenation Event (~2.5 billion years ago), it was not clear how they survived the production of this toxic diradical. One theory proposes that during a "Snowball Earth" period, intense UV irradiation of glacial ice could have built up substantial levels of hydrogen peroxide. Subsequently, release of H_2O_2 during glacier melting, as well as its disproportionation to H_2O and O_2 might have helped drive evolution of oxygen-mediating enzymes (1). Eukaryotes emerged about 2 billion years ago in response to increasing levels of O_2 in the atmosphere. These organisms must have co-evolved numerous mechanisms to protect themselves from oxidative stress, that is, the overproduction of reactive oxygen species (ROS) that would literally bleach critical biopolymers such as proteins and DNA.

In the present day, oxidative stress on the cell's genome stems from both endogenous and exogenous sources that include metabolic intermediates (superoxide and hydrogen peroxide) and products of inflammation (peroxynitrite and hypochlorous acid) (Figure 1). These ROS contribute to daily attack on DNA bases as well as on the sugar-phosphate backbone. Chemical reactions centered on the DNA bases do not generally break the DNA strand, as can happen when hydroxyl radical abstracts a hydrogen atom from the deoxyribose portion of the oligomer, but they are more likely to lead to mutations because base modification can disrupt the normal hydrogen bonding pattern of A:T and G:C base pairs.

Several mechanisms have evolved to seek out and repair changes to the DNA bases, hopefully before replication has sealed in a mutation (Figure 2). In a few cases, the integrity of the base can be restored by direct reversal of the damage. More commonly, minor changes are repaired by the base excision repair (BER) pathway that cleaves the glycosidic bond, releasing the damaged base (2). Further processing is required to remove the deoxyribose so that the appropriate nucleotide can be reinserted with a polymerase, and the ends are then sealed with a ligase. The BER pathway is particularly relevant to oxidative damage, because most reactions involve the addition of only one or two new oxygen atoms to the purine or pyrimidine base. For successively bulkier and more complex damages such as cross-links to other DNA strands or to proteins, more complicated pathways are involved. For example, in nucleotide excision repair (NER), a whole segment of DNA surrounding the lesion is clipped out and then resynthesized using the undamaged strand as a template. For more extensive damage, recombinational repair (not shown) may recruit the other copy of the chromosome. So

dependent are organisms on DNA repair that several disease states have been associated with faulty or inadequate repair due to mutant enzymes (3).

Daily damage to the human genome is summarized in Figure 3 (4); depurination resulting from hydrolytic cleavage of the glycosidic bond is the most common outcome, and the non-instructive abasic site must be repaired. Fortunately, most DNA polymerases pause at abasic sites, permitting repair enzymes to be recruited before a faulty copy of the DNA template is synthesized. Another frequent damage type is deamination of cytosine yielding uracil, which would result in a C-to-T mutation if left unrepaired. Other modifications include formation of pyrimidine-pyrimidine dimers such as the T<>T cyclobutane dimer whose occurrence is highly dependent on exposure to UV light; indeed, it can easily climb to thousands of events per cell per day. Alkylation damage has a number of sources, many of them due to exogenous exposure to inhaled or dietary toxins, but S-adenosylmethionine-mediated (SAM) reactions that normally methylate C, can also erroneously methylate other bases, accounting for additional damage. Alkylation of N7 or N3 of purines enhances the lability of the glycosidic bond leading to abasic sites, therefore requiring repair; alkylation at other sites such as O6 of G can also be mutagenic.

Key among the detrimental damages to DNA bases are the oxidative transformations that occur principally on guanine, the most readily oxidized of the four bases. Measurement of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxoG), a biomarker of oxidative stress, has been intensively investigated, and the current estimate of this lesion under normal cellular conditions is approximately 1 in 10^6 – 10^7 guanosines (5). This means that each cell sees on average more than a thousand oxidized guanines per day. 8-OxoG is not the only product of oxidative damage to the bases; all four bases form adducts with hydroxyl radical, and G and A in particular form cyclic adducts with lipid oxidation products such as malondialdehyde.

Multiple pathways lead to the guanine oxidation product 8-oxoG (Figure 4) including one-electron oxidation of G, addition of hydroxyl radical (from Fenton reactions or ionizing radiation) and reduction of 8-hydroperoxyG, a proposed product of singlet oxygen addition to G, by cellular thiols. Other products of guanine oxidation include imidazolone (dIz) and formamidopyrimidine (Fapy-dG) derivatives. Yet more G oxidation products are produced as secondary oxidation products because of the much lower oxidation potential of 8-oxoG compared to the four natural DNA bases. Our laboratory has investigated the formation of hydantoin products from 8-oxoG oxidation; these comprise two diastereomers of spiroiminodihydantoin (Sp) as well as guanidinohydantoin (Gh) (Figure 5) (6,7). The latter's two diastereomers rapidly interconvert and cannot be studied separately. These products are formed in varying amounts depending on the medium and context; formation of Sp is disfavored in duplex DNA or at pH<6, but it is otherwise an abundant product of G and 8-oxoG oxidation.

Investigation of the mechanism of oxidation of G to form the hydantoin lesions was conducted with isotopically labeled G (8). Using ^{13}C , ^{15}N and ^{18}O heavy isotopes, we were able to determine that the oxygen atom incorporated at C8 was derived from O_2 , while the oxygen atom introduced at C5 originated with H_2O . In this mechanism, water serves as a weak but abundant nucleophile adding to an oxidized quinone form of 8-oxoG. The significance of this study is that it provided us with the broader concept that oxidation of G leads to the formation of an electrophilic species (OG^{ox} in Figure 5), that could be trapped by any number of cellular nucleophiles. Examples of less abundant but better nucleophiles in the cell include the tetraamine spermine and the plethora of DNA-binding proteins which tend to be rich in lysine. Thus, oxidation of G can also trigger DNA-protein covalent cross-linking (9).

8-OxoG is weakly mutagenic in an *E. coli* assay leading to G-to-T transversion mutations. The molecular basis for this mutation is outlined in Figure 6 (10). Although the oxidative damage process that inserts an oxygen atom at C8 of G does not appear to alter the base-pairing properties of G, it does alter the preferred orientation of the base with respect to the glycosidic bond. Thus, 8-oxoG has a higher propensity to rotate into a *syn* conformation rather than the normal *anti* conformation. If it does so during replication, the 8-oxoG base presents a different hydrogen-bonding pattern compared to G, and its complement is A rather than C. If A is misincorporated into the strand opposite 8-oxoG, then there is a chance it will persist until the A-containing strand is replicated, in which case, a T would be placed opposite, in the original position of the G that underwent oxidation.

In contrast, the hydantoins are 99% mutagenic, mispairing with A or G and leading to G-to-T and G-to-C mutations, both of which are common outcomes of oxidative stress (11). The molecular basis of mutation via Gh and Sp is less well established. Presumably, the hydantoin ring resembles a T, albeit a distorted and ring-contracted one, so it may base pair with A, though not as well as the six-membered ring pyrimidine T. Pairing with G might be accomplished via shifting of the hydantoin ring toward the major groove to form a wobble pair; this would better accommodate the sp^3 carbon of the ring and the appended guanidinium (in Gh) or spirocycle (in Sp). (See Figure 6B.) When the hydantoin lesion mispairs with G, another round of replication would replace it with C; overall this would then be a G-to-C transversion mutation.

The high rate of mutagenicity observed for the hydantoin lesions Sp and Gh is due to their severely altered structures, compared to G, and their consequent inability to base pair correctly with C. The good news is that Sp and Gh can be recognized and excised by the base excision repair mechanism using a glycosylase called hNEIL1, the human homolog to bacterial Endonuclease VIII (Nei). In fact, we have found that the hydantoins are more than 100-fold better substrates than any other DNA lesion studied so far (12). Thus, the action of hNEIL1 on a Gh:C or a Sp:C base pair can correct the oxidative damage before mutation. Unfortunately, if replication has already occurred leading to misinsertion opposite the hydantoin (Gh:G, for example), the action of hNEIL1 will serve only to seal in the mutation by removing Gh and allowing its replacement with a new base in that position.

In the timeframe of a human lifespan, we think of mutation as a bad thing—a genetic component of aging and disease. However, without mutation, there would be no adaptation of species to changing environments, *i.e.* no evolution. Mutations can occur randomly due to the constant but low level of errors made by polymerases during replication. Alternatively, mutations can be accelerated due to DNA damage such as oxidation. Presumably, the Great Oxygenation Event of 2.5 billion years ago was responsible for some of the evolutionary pressure to generate proteins that could handle oxidative stress: superoxide dismutase and catalase to reduce the levels of ROS, and DNA repair enzymes to handle the oxidative damage to the genome.

One puzzle that has not been solved about the early evolution of life in an oxygen atmosphere is the question of redox cofactors for metabolic enzymes. The “RNA World” hypothesis suggests that ancient life evolved from the catalytic chemistry of RNA oligomers capable of basic functions like cleavage, ligation, and replication (13). A primitive form of metabolism was also needed, but the modern redox cofactors NADH and FADH₂ were not available. The persistence of the dinucleotide redox cofactors in evolutionary history has strengthened the argument that life evolved from RNA-based chemistry (14), but where did these cofactors come from? How did early RNA catalyze redox reactions without them?

We hypothesize that oxidized purines may have fulfilled this role in an earlier time. The 2-electron oxidation of 8-oxoG is remarkably similar to that of dihydroflavin, and an even closer parallel exists with the redox chemistry of pterins (Figure 7). Biosynthetically, pterins and

flavins are derived from guanosine through a long series of transformations, but the current biosynthesis is presumably a highly evolved pathway that originated from a rudimentary derivative of G that was capable of redox chemistry. Perhaps that was 8-oxoG? In a single chemical step from G to 8-oxoG, the one-electron redox potential of the purine is lowered by 600 mV ($E_{1/2}$ of G is 1.3 V vs. NHE; see Figure 7 for others). Although this is still higher than pterins and flavins, it is nevertheless a huge step in the direction of forming a redox-active ribonucleoside, potentially capable of assisting primitive proteins to develop an oxygen-based metabolism for evolving organisms. We cannot travel back in time to observe early evolution, but current experiments may lend credence to this hypothesis.

Oxidative DNA damage and repair processes are like two ends of a seesaw—when damage increases, repair must be upregulated to keep the genomic mutation rate in balance. Otherwise, oxidative stress could become catastrophic for the cell, or at a minimum, may cause permanent changes in the genome. On the positive side, the evolution of the species depends on mutation, and indeed the evolution of life as we know it may have depended on oxidative purine chemistry to utilize the oxygen atmosphere to its advantage.

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Figure 1.

Reactive oxygen species, endogenously or exogenously produced, are major contributors to DNA damage leading to disease.

Figure 2.

Three of the DNA repair pathways. Direct reversal of damage is rare. Base excision repair (top loop) in which the damaged base is cleaved from the ribose, then the ribose is removed leaving a gap filled in by a polymerase (pol) and religated with a ligase. Nucleotide excision repair (bottom loop) is similar to BER except a longer fragment surrounding the damage is removed.

Figure 3.
Structures of common DNA lesions and their frequency of occurrence.

Figure 4. Pathways leading to 8-oxoG include ionizing radiation ($HO^{\cdot}$) and one-electron oxidation.

Figure 5.
Formation of hydantoin products from further oxidation of 8-oxoG.

Figure 6.

A. Structural basis of G-to-T transversion mutations via the 8-oxoG:A mispair. B. G-to-C mutations might occur via Gh formation that pairs with G, by analogy to T:G wobble pairs.

Figure 7.
Analogy between redox chemistry of 8-oxoG and pterins and flavins.