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8-Oxo-7,8-dihydroguanine, friend and foe: Epigenetic-like regulator versus initiator of mutagenesis

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Abstract

A high flux of reactive oxygen species during oxidative stress results in oxidative modification of cellular components including DNA. Oxidative DNA "damage" to the heterocyclic bases is considered deleterious because polymerases may incorrectly read the modifications causing mutations. A prominent member in this class is the oxidized guanine base 8-oxo-7,8-dihydroguanine (OG) that is moderately mutagenic effecting G→T transversion mutations. Recent reports have identified that formation of OG in G-rich regulatory elements in the promoters of the *VEGF, TNFα*, and *SIRT1* genes can increase transcription via activation of the base excision repair (BER) pathway. Work in our laboratory with the G-rich sequence in the promoter of *VEGF* concluded that BER drives a shift in structure to a G-quadruplex conformation leading to gene activation in mammalian cells. More specifically, removal of OG from the duplex context by 8-oxoguanine glycosylase 1 (OGG1) produces an abasic site (AP) that destabilizes the duplex, shifting the equilibrium toward the G-quadruplex fold because of preferential extrusion of the AP into a loop. The AP is bound but inefficiently cleaved by apurinic/apyrimidinic endoDNase I (APE1) that likely allows recruitment of activating transcription factors for gene induction. The ability of OG to induce transcription ascribes a regulatory or epigenetic-like role for this oxidatively modified base. We compare OG to the 5-methylcytosine (5mC) epigenetic pathway including its oxidized derivatives, some of which poise genes for transcription while also being substrates for BER. The mutagenic potential of OG to induce only ~one-third the number of mutations (G→T) compared to deamination of 5mC producing C→T mutations is described. These comparisons blur the line between friendly epigenetic base modifications and those that are foes, i.e. DNA "damage," causing genetic mutations.

Keywords

8-Oxo-7,8-Dihydroguanine; Base Excision Repair; Epigenetics; G-Quadruplex; Oxidative Stress; Mutagenesis

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1. Introduction

Reactive oxygen species formed during oxidative stress are electron deficient and readily oxidize proteins, lipids, RNA, and particularly DNA. Oxidative modification of the genomic DNA bases is well documented and can result in mutations responsible for initiation of a number of diseases [1]. The guanine (G) heterocycle is the most susceptible of the four DNA bases to oxidation leading to many products [2]. Chief among these oxidatively modified products is 8-oxo-7,8-dihydroguanine (OG; Fig. 1). Cellular levels of OG in the genome are routinely monitored as a biomarker to assess the extent of oxidative stress to which a cell has been exposed [3]. Moreover, OG is moderately mutagenic, if not repaired, causing G→T transversion mutations that are thought responsible for initiating and driving some cancers [1]. These mutations are a consequence of OG base pairing with A on the Hoogsteen face rather than C on the Watson-Crick face [4]. To counteract mutations from damaged DNA nucleotides, an elaborate DNA repair system has evolved to return modified sites back to the original canonical nucleotides [5]. Repair of OG is achieved by base excision repair (BER) that initiates removal of OG when base paired with C by the action of 8-oxoguanine glycosylase 1 (OGG1) in mammals (Fig. 1); in contrast, when OG is incorrectly base paired with A, MutY DNA glycosylase (MUTYH) removes the A allowing a second chance for a polymerase to insert C opposite OG for further action by OGG1 [5]. Following removal of OG by OGG1, an abasic site (AP) is formed that is a substrate for apurinic/apyrimidinic endoDNase I (APE1) to cleave the 5′-phosphodiester linkage yielding a nick in the DNA (Fig. 1) [5]. The repair process is completed by polymerase β (POLB) that removes the sugar fragment at the nick site followed by inserting the correct G nucleotide, and finally ligase (LIG) seals the nick to return the DNA back to its native state (Fig. 1) [5]. This dynamic process of G oxidation to OG followed by DNA repair has been estimated to occur up to 10^5 times per cell per day [6].

The long-standing view has been that OG is mutagenic and detrimental to cellular processes such as transcription. For instance, the presence of OG in template strands can stall the advancement of RNA pol II [10], and initiation of OG repair causes polymerases to stop [11], thus ascribing a role to OG as a transcriptional repressor. However, there are a few notable examples of oxidative stress leading to increased OG formation in the genome in tandem with increased gene expression. This has been documented in livers from mice with infection-induced colitis [12], and rat pulmonary artery endothelial cells exposed to hypoxic conditions [13]. Observations like these led our laboratory and others to inspect how the *VEGF* [14], *TNFα* [15], and *SIRT1* [16] genes respond when G is oxidized to OG in their promoters. The most interesting finding in these cellular studies showed that OG can increase gene transcription via the BER pathway [14–16]. These results identify an intertwining of DNA repair with gene activation that is a phenomenon gaining appreciation [17]. Therefore, oxidative modification of G to OG may have regulatory and possibly epigenetic-like features in cells that are responding to oxidative stress. This perspective will discuss the discovery that OG can stimulate transcription via BER activity. These results provide the background for a comparative discussion between OG as a possible epigenetic-like DNA modification vs. the traditional 5-methylcytosine (5mC) epigenetic modification. Additionally, the ten-eleven translocation (TET) proteins oxidize 5mC in a stepwise fashion

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to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (Fig. 1) in genes poised for activation by BER removal after being silenced by 5mC [18–21]. The observation of oxidative modification to DNA bases in the form of OG or oxidized 5mC highlights a possibility that base oxidation is a DNA-based mechanism for gene activation. Finally, the ability of OG to regulate gene expression vs. its ability to cause mutations will be discussed.

2. Initial reports that OG is epigenetic-like

A few initial reports proposed that OG, if present in key regions of the genome, could impact cellular processes. For instance, synthetic oligonucleotides with OG in protein transcription factor binding sequences found this modification negatively impacted factor binding affinity. This effect was demonstrated in the consensus sequences of specificity protein 1 (SP1) [22], nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [23], and CAMP responsive element binding protein 1 (CREB) [24]. The work with the CREB transcription factor in the Strauss laboratory led them to propose that OG might be epigenetic by decreasing protein binding resulting in OG as a transcriptional repressor [24]. The Olinski laboratory quantified OG in heterochromatin vs. euchromatin from porcine thymus DNA to find that the transcriptionally active euchromatin DNA harbored more OG [25]. Their observation of OG concentrations varying throughout the genome led them to speculate that OG might be an epigenetic modification. Lastly, Park, et al. developed a method to demonstrate that G oxidation to OG could occur site specifically *in vivo* under oxidative stress conditions leading them to propose OG as an epigenetic modulator [26]. These observations of OG as a regulatory modification (i.e., epigenetic-like) were all lacking in cellular experiments demonstrating G oxidation to OG can form in critical regions of the genome and impact transcription.

3. OG activates mRNA synthesis by facilitating promoter G-quadruplex formation

The vascular endothelial growth factor A (*VEGF*) gene harbors a G-rich promoter element critical for regulation of mRNA synthesis [27]. The G-rich element is located between positions −86 and −56 relative to the transcription start site (TSS) in the coding strand (Fig. 2A); further, this region is bound by three equivalents of the SP1 transcription factor [27]. Cellular regulation of *VEGF* by SP1 has been documented, and oxidative stress results in less SP1 binding followed by up-regulated transcription [27]. Moreover, this G-rich element is a potential G-quadruplex sequence (PQS) as demonstrated by Hurley and co-workers with the ability to adopt a G-quadruplex (G4) structure for regulation of transcription [28, 29] (Fig. 2A). Additionally, the Gillespie laboratory found hypoxia-induced oxidative stress increased transcription of the *VEGF* gene, in addition to OG formation in the vicinity of the gene promoter, likely near the PQS [13, 30]. These observations prompted us to inquire if oxidation of G to OG in the G-rich promoter element of *VEGF* could facilitate activation of transcription, whether BER is involved in the reactivation process, and whether there is a role for the G4 structure in gene induction.

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To test our hypothesis, we developed a luciferase reporter plasmid with the *VEGF* PQS regulating the Renilla luciferase gene [14]. The system developed allowed synthetic incorporation of OG within the G-rich sequence with single-nucleotide precision. To guide selection of appropriate sites for modification, we first oxidized the *VEGF* PQS in a short oligonucleotide folded as a G4 [31] or in the duplex context with a reactive oxygen species (ROS) found in inflammation to identify G sites readily oxidized (Fig. 3) [32]. Armed with the knowledge of the most reactive Gs in the *VEGF* promoter sequence, we then synthetically incorporated OG into the plasmid at specific sites in the promoter (Fig. 2A red Gs) [14]. Next, transfection of the synthesized reporter plasmids into mouse embryonic fibroblasts (MEFs) revealed that the OG-containing plasmids produced >2.5-fold more luciferase protein than a wild-type (WT) plasmid without OG (Fig. 2B). Furthermore, luciferase expression was always increased regardless of where the OG was located (Fig. 2B). Gene induction with the reporter system was verified to produce a similar increase in luciferase expression in glioblastoma cells. Demonstration that BER of OG was essential in the initiation of gene activation was accomplished by repeating the studies in MEFs that had OGG1 knocked out (Fig. 2B $OGG1^{-/-}$ MEFs). When OGG1 was absent, the presence of OG in the reporter plasmid did not yield an increase in expression relative to the WT control. The null result in $OGG1^{-/-}$ MEFs highlights a critical role for OGG1 in the process of gene activation when OG is in the coding strand of the *VEGF* PQS element.

When OGG1 removes OG from the DNA in the cellular context, the product is an AP site that is subsequently the substrate for the next enzyme in the BER process, APE1 (Fig. 1) [11, 33]. Accordingly, we next synthesized catalytically competent, yet stable, AP analogs (tetrahydrofuran, F) in the reporter plasmids and then transfected them into MEF and glioblastoma cells. These AP-modified plasmids were studied to determine the importance of an AP and of APE1 in the gene activation process. First, when an AP analog was present in the reporter system, the luciferase gene was induced >4-fold, a value somewhat higher than that obtained for OG (Figs. 2B and 2C) [14]. Second, demonstration that APE1 was essential for gene induction was achieved by transfecting cells with AP analog-containing plasmids and knocking down APE1 with siRNAs. As siRNAs specific to APE1 were titrated into cells transfected with AP-analog containing plasmids, the level of luciferase expression decreased with a dose response (Fig. 2C). These experiments concluded that the AP site resulting from OG release from the DNA is critical for gene induction with APE1 playing a major role in the process. The activity of APE1 expands beyond it being a key player in BER to include interactions via its redox-effecter factor-1 (Ref-1) domain with protein factors such as HIF1-α, STAT3, and CBP/p300 that promote gene transcription [13, 34]. Future studies will begin to unravel more details of the activating transcription factors involved in the gene induction process when OG is processed by BER in the *VEGF* gene promoter region.

Beyond the importance of the BER process for activation of transcription, we found that the ability of the *VEGF* PQS to possibly adopt a G-quadruplex fold is also essential for gene activation. G-Quadruplexes are structures that can fold in DNA sequences with four or more contiguous runs of >3 Gs with small intervening sequences forming loops between the G runs [35]. These structures diverge from the native B-form of DNA because they fold around cellular K^+ ions to G tetrads held together by four G:G Hoogsteen base pairs (Fig. 3). In

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DNA, generally three or more G tetrads stack to adopt unique four-stranded folds that are structurally different than B-form DNA (Fig. 3) [36, 37]. Protein interactions with Bform and G4 DNA differ resulting in redirecting the downstream signals [38]. The ability of G4 structures to regulate transcription was best demonstrated by the Hurley laboratory in the *c-MYC* gene [38]. Global cellular confirmation of PQS to adopt G-quadruplex folds and alter transcription was recently demonstrated by the Balasubramanian laboratory through a combination of G4 ChIP-Seq and RNA-Seq experiments in human cells [39]. The *VEGF* PQS was found to adopt a parallel-stranded G4 structure on the basis of NMR structural analysis (Fig. 3) [31]. These studies and many others not referenced provide a solid background for us to consider the possibility that a G4 structure may exist in the *VEGF* PQS when OG is present and aid in the activation process.

Comparisons were made between plasmids containing OG in either the PQS sequence, or one judiciously mutated to be incapable of G4 formation, while still retaining the ability to be bound by the SP1 transcription factor [14]. The comparative studies found that the G4 structure was essential to induce transcription; in contrast, the G4 negative sequence provided no signal enhancement relative to the control. These results support the importance of the G4 fold in gene induction. Our previous analyses found a significant number of promoter PQSs possess additional G tracks flanking the core G4 structure (Fig. 2A) [32]. We proposed when a core G4 sequence is damaged leading to the unraveling of a G tetrads and the loss of global structure, the 5th G run is recruited as a “spare tire” to maintain the G-quadruplex fold (Figs. 2D and 3). The recruitment of the 5th G run allows extrusion of the modification into a large loop to achieve G-quadruplex folding; furthermore, the plasticity of these five G-track sequences allow BER enzymes to bind the modifications in the large loop. The Burrows and Wallace laboratories found that without the 5th domain present, DNA repair initiation was abolished in the *VEGF* PQS (Fig. 2A and D) [32, 41]. As a final study, comparisons were made between transfected plasmids containing OG in the native sequence with all five G tracks (G5) present to a modified sequence with only the essential four core G tracks (G4). The results of these studies led to the conclusion that both sequence contexts yield induction of transcription when OG is present, but the expression was significantly greater for the G5 sequence [14]. This observation supports an important role for the 5th domain in achieving the maximal transcriptional increase when OG is present.

These results led to the following proposed mechanism for transcriptional activation when OG is present in the coding strand of the *VEGF* promoter PQS (Fig. 2D) [14]. The G-rich PQS element renders this site highly susceptible to oxidative modification of G to OG in the duplex context. This oxidation yields an OG base paired with C that has a negligible impact on the B-form DNA structure (Fig. 3). Oxidation of G to OG allows recruitment of OGG1 for removal of OG to yield an AP. On the basis of initial biophysical studies, the AP results in melting of the duplex to unmask the more thermodynamically favorable G-quadruplex structure (Fig. 2D) [14]. The G-quadruplex fold is favored because the 5th G track allows extrusion of the damaged G run into a large loop while maintaining the fold. The AP site is then presented to APE1 and bound by this protein; consequently, the reaction kinetics are highly attenuated by the G4 structure prolonging APE1 binding [42] and aiding in the possible recruitment of other activating transcription factors for gene induction (e.g., HIF1-α) [13]. These initial studies identify an intertwining of G oxidation in PQS elements to

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provide a structural switch for recruitment of BER proteins to activate transcription (Fig. 2D). Furthermore, we identify the AP site and APE1 as central players in the gene activation process initiated by G oxidation to OG in the PQS of the coding strand of the *VEGF* gene.

In our publication of these results [14], the concept of a PQS structural switch was also demonstrated in a PQS found in the coding strand of the *NTHL1* DNA repair gene. The presence of OG in the *NTHL1* PQS yielded a >4-fold transcriptional enhancement. This example expands the possibility that the mechanism proposed may be a more general phenomenon for gene activation under oxidative stress conditions. The *VEGF* and *NTHL1* results set the stage for many future inquiries. For instance, G4 ChIP-Seq in human skin cells found ~10,000 PQSs responsible for gene induction [39]; are any of these genes also activated by oxidative modification of G to OG in the PQSs? The strand distribution of PQSs is nearly equal on the coding and template strands [43], and so far, the PQS structural switch leading to gene induction has only been reported for sequences in the coding strand. Will the gene output be different when the PQS is in the template strand vs. the coding strand? Structural studies have found G-quadruplex sequences adopt many different types of folded structures [36, 37]. How does G4 folding (parallel, anti-parallel, or hybrid) impact the structural switch? These interesting questions should be addressed in the near future.

The proposal of an AP being processed by APE1 as the key step for gene activation begs the question: do other oxidatively modified DNA bases that are processed to yield APs also impact transcription? There exist a few notable examples of related modifications to study. First, the 4-electron oxidation products of G (or 2-electron oxidation products of the highly redox sensitive OG) are 5-guanidinohydantoin and spiroiminodihydantoin (Gh and Sp; Fig. 1) [7–9], both of which are excellent substrates for the NEIL glycosylases [44]. Processing of Gh and Sp by the monofunctional NEIL3 glycosylase yields an AP (Fig. 1) [45]; in contrast, NEIL1 or NEIL2 are proposed to be bifunctional glycosylases leading directly to a strand break [44]. The details of hydantoin processing in PQSs and the subsequent impact on transcription will guide a better understanding of the substrate and glycosylase requirements to activate transcription. Another interesting modification for study is C deamination to uracil (U) because in mammals, U is removed by the monofunctional glycosylases UNG and SMUG1 to yield an AP for processing by APE1 [46]. Future inspection of these modifications and others will be fascinating for expanding our knowledge of the PQS switching mechanism activated by BER to induce transcription.

4. Other examples of OG as an epigenetic-like DNA base modification

Boldogh and co-workers recently provided an additional observation of possible OG formation in promoters followed by OGG1 recruitment to induce transcription [15]. Briefly, their studies in HEK 293 and MEF cells found tumor necrosis factor-alpha (TNFα) possibly induced G oxidation to OG upstream of NF-κB consensus sequences in pro-inflammatory genes (e.g., TNFα). The result of OG formation was recruitment of OGG1 followed by NF-κB protein to up-regulate transcription of these genes. These results provide strong additional support for the oxidatively modified G residue, OG, as a regulatory modification by facilitating BER and transcriptional regulation networks working together on promoter sequences. The Tell laboratory studied HeLa cells under oxidative stress conditions to

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propose G oxidation to OG in the negative calcium responsive elements (nCaRE) in the promoter of the sirtuin-1 (*SIRT1*) gene [16]. Their work led them to propose that OGG1 removed OG in the nCaRE sequence to yield an AP followed by APE1 binding to the site. Next, APE1 functions in tandem with Ku70 and RNA Pol II to increase *SIRT1* transcription; more importantly, their work, like ours [14], supports both AP and APE1 as key elements for gene activation associated with OG formation in gene promoters. The studies in our laboratory with the VEGF promoter PQS are the only experiments to date demonstrating that OG, when site-specifically found in the coding strand of a regulatory element, can upregulate transcription.

Not all examples of OG formation in cellular DNA lead to enhanced transcription. The Hanawalt laboratory found OG in template strands slightly inhibited advancement of RNA pol II and recruits the transcription-coupled repair machinery slowing transcription [10, 47]; additionally, this work concluded that the AP site generated by OGG1 release of OG blocked transcription and initiated transcription-coupled repair. The Khobta laboratory identified that OG is a barrier to transcription when located in either the coding or template strand in a gene coding region [11, 48]. Further, OG is only a transcriptional barrier after conversion to an AP site by OGG1. The experiments in our laboratory and the others demonstrate that OG can modulate transcription, and the direction of the modulation is dependent on the context in which OG is located within the genome. Further investigation into sequence contexts, such as PQSs, outside of promoters is also warranted to advance our knowledge of the impact OG has during synthesis of an entire mRNA sequence.

5. How does OG fit into the epigenetic landscape?

Many of the studies described propose that OG is an epigenetic modification [14, 15, 24–26]. Can OG actually be classified as an epigenetic mark, and if so, how does it fit into a classification system that typically involves heritability? In the traditional 5mC epigenetic system, there has emerged a clear picture of the protein writers, readers, and erasers for modifications on the global genome [18–21]. A wealth of data has led to a better understanding of how these modifications change in the genomes of different cell types, how these modifications are heritable from mother to daughter generations, and how these modifications impact gene activity and expression. This knowledge forms the modern epigenetic definition [49]. Some of these features with respect to OG are clear while others need further inquiry before the mechanisms are understood.

For 5mC, there exist DNA methyltransferases (DNMT) to write the modifications site specifically into the genomes (Fig. 1) [18]. It is well established that OG is formed in genomic DNA by direct oxidation of G via ROS [2], or indirectly written by remote oxidation and electron transfer through the DNA π stack to induce G oxidation [50]. These mechanisms generally effect oxidation of a 5′ G in the sequence context 5′-GpG-3′ that provides a pathway to obtain OG in a specific dinucleotide context, just as 5mC occurs in the 5′-CpG-3′ context. The ability of ROS to selectively modify critical Gs for cellular regulation is challenging to envision. Work by Perillo, et al. found that chromatin remodeling could induce region-specific oxidation of G to OG in the *BCL-2* promoter of MCF7 cells for gene activation [51]. They identified that the flavin-dependent lysine-

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specific demethylase 1A or 1B (LSD1 or LSD2) remodelers generated H_2O_2 in the vicinity of the genome for oxidation of G to OG, most likely by the Fenton reaction [52]. More interestingly, the Perillo, et al. work documented that OGG1 and BER were essential for *BCL-2* gene induction [51]; in our studies [14], we note the region oxidized in *BCL-2* is a PQS proposed to be involved in regulation of this gene [38], and therefore, the activation mechanism Perillo, et al. observed may have functioned through a mechanism similar to our proposal for *VEGF* (Fig. 2D) [14]. Thus, one possibility is that chromatin remodeling by LSD1/2 can induce region-specific G oxidation to OG for gene regulatory purposes, although the LSD1/2 mechanism relies on diffusion-controlled delivery of the H_2O_2 oxidant to the regulatory site of G oxidation. Evolution of this approach to write OG into the genome for gene activation remains less refined compared to a direct protein-catalyzed oxidation mechanism. Future exploration to better understand the details of writing OG into the genome are warranted; for example, are there protein writers to site-specifically install OG in the genome?

The presence of OG in genomic DNA is well established by nuclease and phosphatase digestion of cellular DNA followed by mass spectrometric quantification; however, this approach does not allow knowledge of the sequence or region in which OG is located. Thus, a major hurdle along the way to elucidating the role of OG in gene regulation will be the development and implementation of genome-level OG sequencing. Many laboratories have developed antibody-based OG sequencing that provides a low resolution sequence map of OG (~10–1000 kbp) [53, 54]. Recently, our laboratory developed an OG sequencing approach with single-nucleotide resolution implemented on plasmid DNA [55], in addition to an OG sequencing method with ~0.15-kbp resolution (i.e., OG-Seq) that was implemented on the mouse genome [56]. Expansion of sequencing studies, ideally at single-nucleotide resolution, to different cell types under a variety of conditions (i.e., different stressors or cell states) will be essential to the determination of the sequences in which OG is preferentially formed, whether these sequences and regions change based on cellular conditions, and whether they are gene regulatory regions.

The 5mC modification is read by methyl-CpG-binding proteins to silence transcription. Upon oxidation of 5mC to 5hmC, 5fC, or 5caC by TET enzymes in gene regulatory regions, the sites become poised for activation (Fig. 1) [18]. Oxidative modification of 5mC recruits a new set of reader proteins, some of which are involved in DNA repair, such as TDG, NEIL1, and NEIL3 [19]. These DNA repair readers also constitute the erasing mechanism for 5mC from the genome (Fig. 1, TDG) [19]. The recruitment of BER proteins to oxidized 5mC is similar to the recruitment of OGG1 by OG, providing a fascinating link between these two systems involved in gene activation. Both TDG binding and removal of 5fC or 5caC and binding and removal of OG by OGG1 yield an AP product (Fig. 1) [11, 57]. The AP product recruits APE1 to continue BER in both cases [57]. From our studies, an AP in a coding strand G-quadruplex provides the structural switch that stalls APE1, possibly recruiting additional factors and leading to activation of transcription. Are oxidized base modifications such as 5fC, 5caC, and OG gatekeepers for site-specific introduction of AP, in which the AP facilitates gene activation in both cases? In the case of OG, OGG1 is the best established reader protein; however, systematic studies to identify OG-specific readers have yet to be conducted. It is very possible that for OG, the reading and erasing mechanisms are coupled

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by overlapping DNA repair and gene activation functions. This coupling concept allows cells to complete two necessary tasks during oxidative stress: 1) Repair of oxidatively modified DNA bases, and 2) alteration of the cellular phenotype in response to oxidative stress.

Whether OG is truly epigenetic or a regulatory modification is not clear. For instance, heritability of OG from mother to daughter generations is not known and will require deep inquiry to answer this question. Methylation of C is well established to guide cellular differentiation during embryonic development for long-term cellular information storage [19]; in contrast, OG appears to be a modification allowing cells a rapid response pathway during oxidative stress. Beyond the toxicology of OG formation during oxidative stress, OG formation may be important during embryonic growth. Take the following example: *VEGF* is essential for vascularization; as embryos grow they become slightly hypoxic leading to the need to vascularize [58]. Does OG formation in the *VEGF* PQS drive this process via a mechanism such as we have outlined (Fig. 2D) [14]? If this is possible, it would provide a link between OG formation in the genome and cellular development. It is hard to envision that OG would possess the long-term information potential that 5mC has because OG is directly recognized by BER [5], while 5mC must be modified by TET proteins to become a substrate for BER [19]. Thus, OG may fit into the epigenetic landscape as a DNA modification allowing cells to adapt and respond to changes important on the short term, such as oxidative stress, while 5mC is relegated to longer term cellular information storage. Is this sufficient to define OG as epigenetic? Future studies and more discussion on this topic will craft how we best describe OG and other base modification in the future.

6. OG as a friend and foe

The experiments and discussion so far have acknowledged, against conventional wisdom, OG may be a cellular friend by facilitating gene activation via a DNA repair mechanism in response to oxidative stress [13–16]. However, the toxicological aspects of OG cannot be ignored. Cells deficient in BER of OG accumulate G→T transversion mutations highlighting the mutagenic potential of OG [59]. The *VEGF* example provides a case in which OG formation driving gene expression can be viewed as a foe, or unwanted outcome. For instance, as solid tumors grow, they become hypoxic inducing oxidative stress leading to *VEGF* activation and vascularization allowing tumor growth and metastasis [58]. This process is good for the tumor and bad for the organism. This underscores the importance of context for these modifications in regulating biological processes. This is even true for 5mC in which cancer cells hijack the methylation systems to change cellular phenotype to benefit the cancer at the expense of the organism [60].

Lastly, we compare the mutagenicity of OG vs. 5mC. The mutagenic potential of OG to effect G→T transversion mutations is well established (Fig. 4) [4]. The transversion mutations result from the facile formation of OG:A base pairs by polymerase activity that upon a second polymerase bypass, inserts a T opposite A to occupy the original position of G (Fig. 4) [4]. The frequency for OG to cause this mutation signature is estimated at ~1% [61]. There exists a steady state level of ~30,000 OGs (mouse embryonic stem cell) [62] that could result in ~300 mutation events under replicating conditions (Fig. 4). The epigenetic

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mark 5mC can also cause C→T transition mutations upon deamination of 5mC to T (Fig. 4) [63]. The C→T transition rate for 5mC is estimated at ~2×10^{-3}% [63]. There exist ~50 million 5mCs (in mouse embryonic stem cells) [62] that could lead to ~1,000 mutations (Fig. 4). Therefore, on the basis of this estimate, the epigenetic mark 5mC induces ~3-fold more mutations than OG. This is consistent with recent deep genome sequencing experiments that identified more C→T mutations than G→T [64]. The line that differentiates DNA modifications as mutagenic vs. epigenetic is blurred, and that should result in a reevaluation of modifications considered mutagenic. The former "DNA damage" bases 5-hydroxymethyluracil and 6-methyladenine have recently been reconsidered as possible epigenetic modifications [62, 65], and recent findings support OG joining this list [13–16]. The context in which these modifications exist, especially OG, is exceedingly important in defining whether they are a friend or foe to biological processes and the overall organism: coding vs. template strands, promoter vs. transcribed regions, G-quadruplexes vs. CpG islands—all appear to modulate the roles played by base modifications in cellular function and survival.

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Abbreviations

AP	abasic site
APE1	apurinic/apyrimidinic endoDNase 1
BCL2	B-cell lymphoma 2
BER	base excision repair
CBP	CREB binding protein
ChIP-Seq	chromatin immunoprecipitation assay with sequencing
c-MYC	V-myc avian myelocytomatosis viral oncogene homolog gene
CREB	CAMP responsive element binding protein 1
5caC	5-carboxylcytosine
DNMT	DNA methyltransferase
5fC	5-formylcytosine
F	tetrahydrofuran
G4	G-quadruplex
G	guanine

Gh	5-guanidinohydantoin
HIF1-α	hypoxia inducible factor 1 alpha
5hmC	5-hydroxymethylcytosine
Ku70	protein encoded by the x-ray repair cross complementing 6 gene
LSD1-2	lysine demethylase 1A and 2A
LIG	ligase
5mC	5-methylcytosine
MCF-7	Michigan Cancer Foundation-7 cell line
MEF	mouse embryonic fibroblast
MUTYH	MutY DNA glycosylase
nCaRE	negative calcium response elements
NEIL1-3	endonuclease VIII-like 1–3
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NTHL1	Nth-like DNA glycosylase 1 gene
OG	8-oxo-7,8-dihydroguanine
OGG1	8-oxoguanine glycosylase 1
OGG1$^{-/-}$-MEF	mouse embryonic fibroblast with OGG1 knocked out
OG-Seq	8-oxo-7,8-dihydroguanine sequencing
p300	E1A binding protein P300
POLB	polymerase β
PQS	potential G-quadruplex sequence
Ref-1	redox effector factor 1
RNA pol II	RNA polymerase II
ROS	reactive oxygen species
SIRT1	sirtuin 1 gene
SMUG	single-stranded-selective monofunctional uracil-DNA glycosylase 1
Sp	spiroiminodihydantoin

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SP1	specificity protein 1
STAT3	signal transducer and activator of transcription 3
TDG	thymine-DNA glycosylase
TNFα	tumor necrosis factor α gene
U	uracil
UNG	uracil-DNA glycosylase
VEGF	vascular endothelial growth factor A gene
WT	wild type

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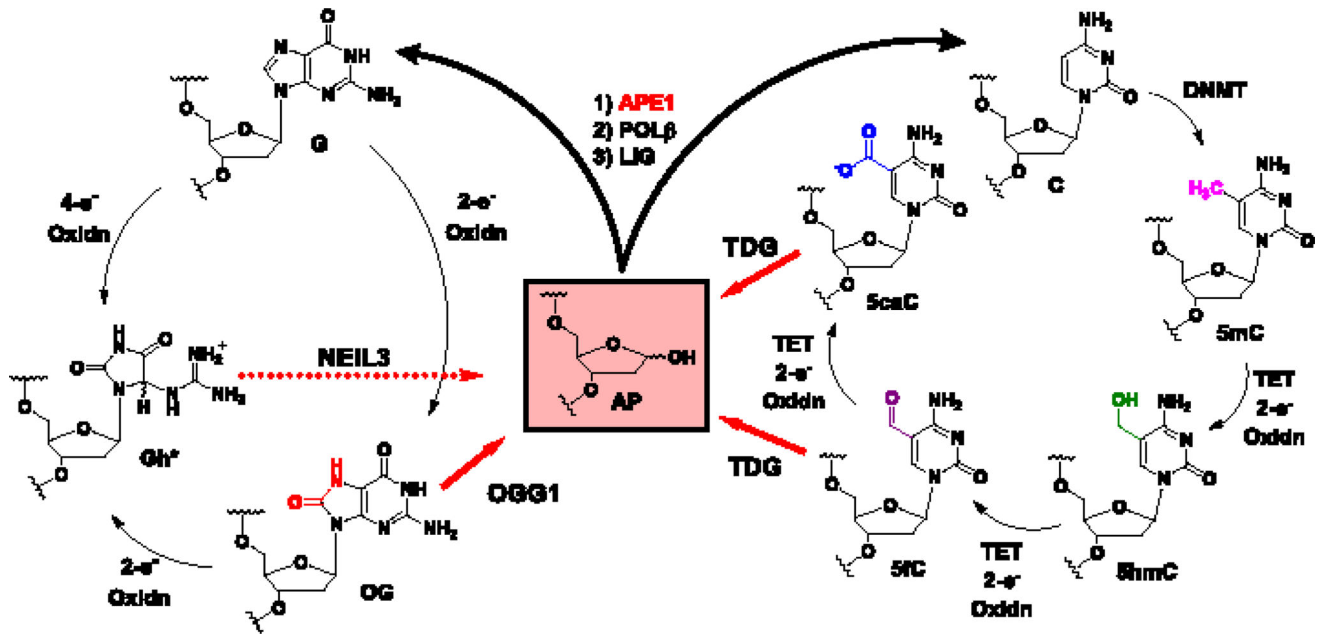

Fig. 1.

Comparison of the G oxidative modification cycle with the C methylation and oxidative modification cycle to illustrate the centrality of the abasic site (AP) to return the sequence back to the original active state. *For the sake of brevity, the 4-electron oxidation product of G, or 2-electron oxidation product of OG yielding 5-guanidinohydantoin (Gh) is shown; the other 4-electron product spiroiminodihydantoin (Sp) is not shown [7]. The yields of Gh and Sp show strong dependency on the reaction conditions and context, favoring Gh in duplex DNA oxidations or reactions at pH < 6 and favoring Sp in single-stranded and G-quadruplex DNA oxidations or reactions at pH > 7 [8, 9].

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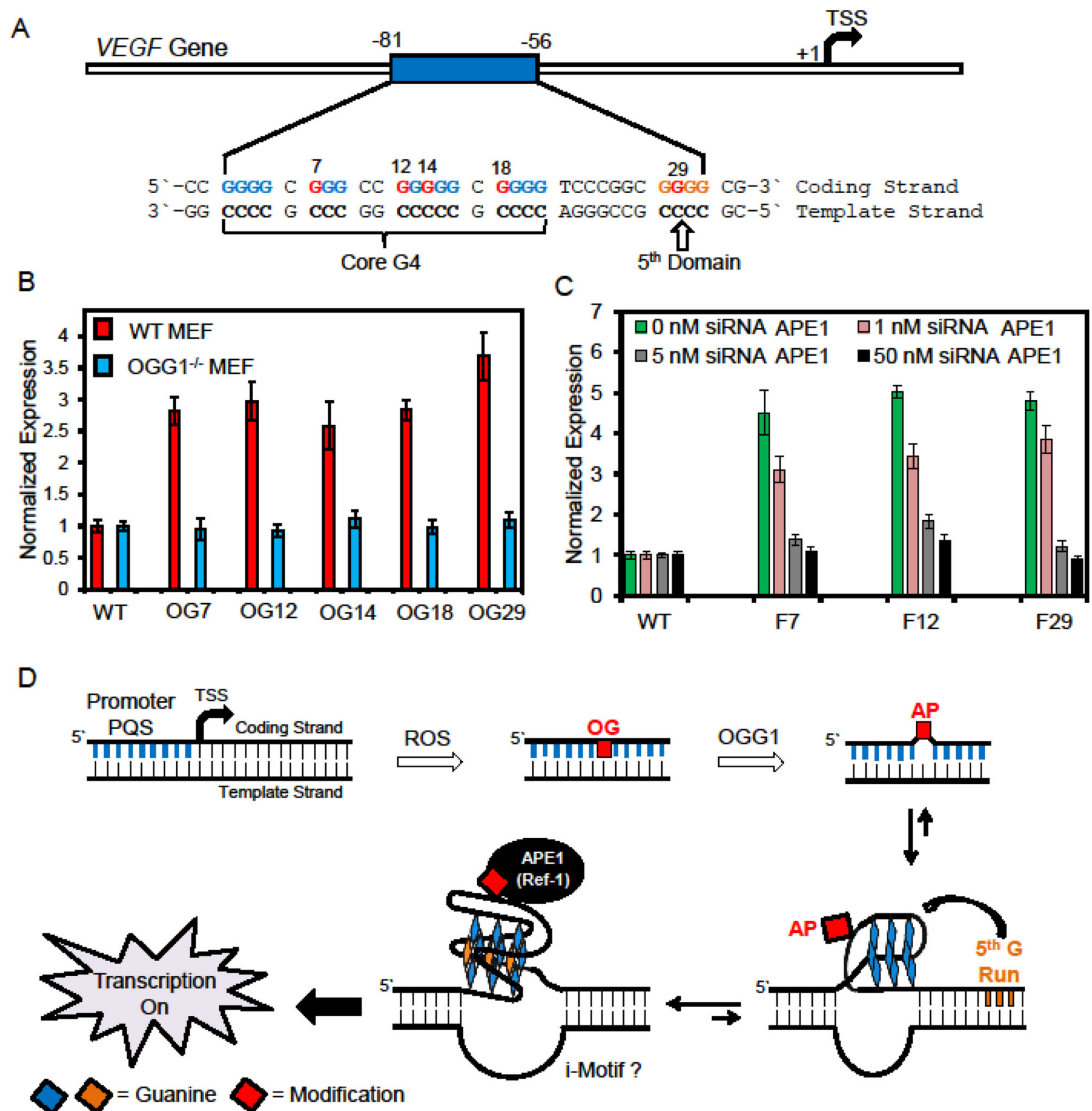

Fig. 2.

Sequence for the PQS in the coding strand of the *VEGF* gene that upon oxidation of G to OG provides a substrate for BER that unmasks the G-quadruplex for gene induction. (A) The sequence of the G-rich element in the *VEGF* promoter. The Gs marked in red are sites in which OG was synthetically incorporated to demonstrate the proposed pathway in part D [14]. (B) Data illustrating the presence of OG in the *VEGF* promoter increased luciferase expression by >2.5 fold in MEF cells, and knocking out OGG1 results in the signal remaining unchanged relative to the wild type (WT) plasmid. (C) Utility of APE1-specific siRNAs in glioblastoma cells provided a dose response impact on luciferase expression. The

data in panels B and C demonstrate OGG1 and APE1, respectively, are required for gene induction when OG is present in the *VEGF* promoter PQS. These data were adapted from the original publication [14]. (D) Proposed pathway for oxidation of the PQS to yield OG and guide the BER process by unmasking the G-quadruplex for gene activation, thus illustrating an intertwining of DNA repair and transcriptional induction.

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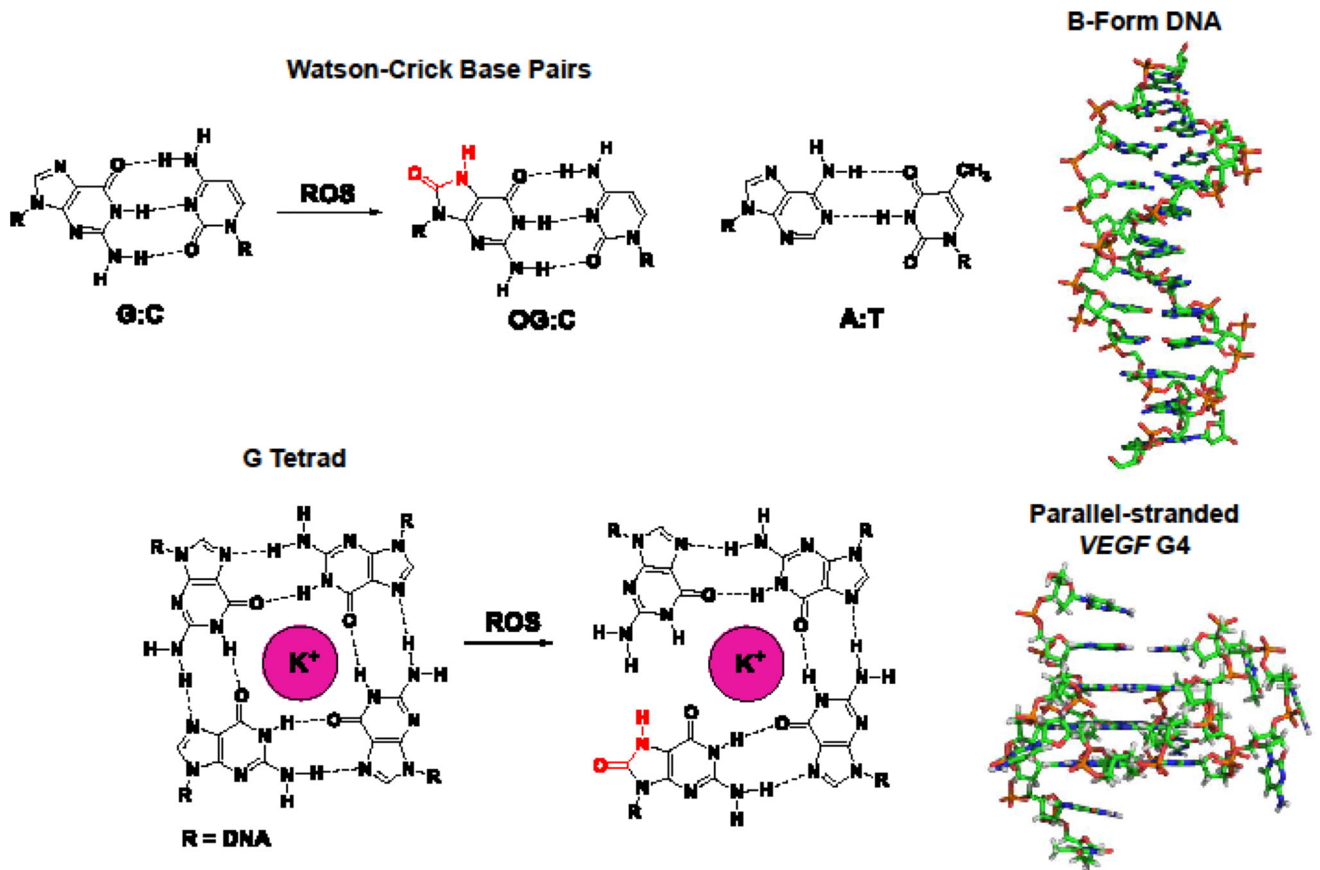

Fig. 3.

Structural comparison and base pairing properties of B-form and G4 DNA. The B-form DNA structure was derived from pdb 1BNA [40], and the *VEGF* G4 structure was derived from pdb 2M27 [31].

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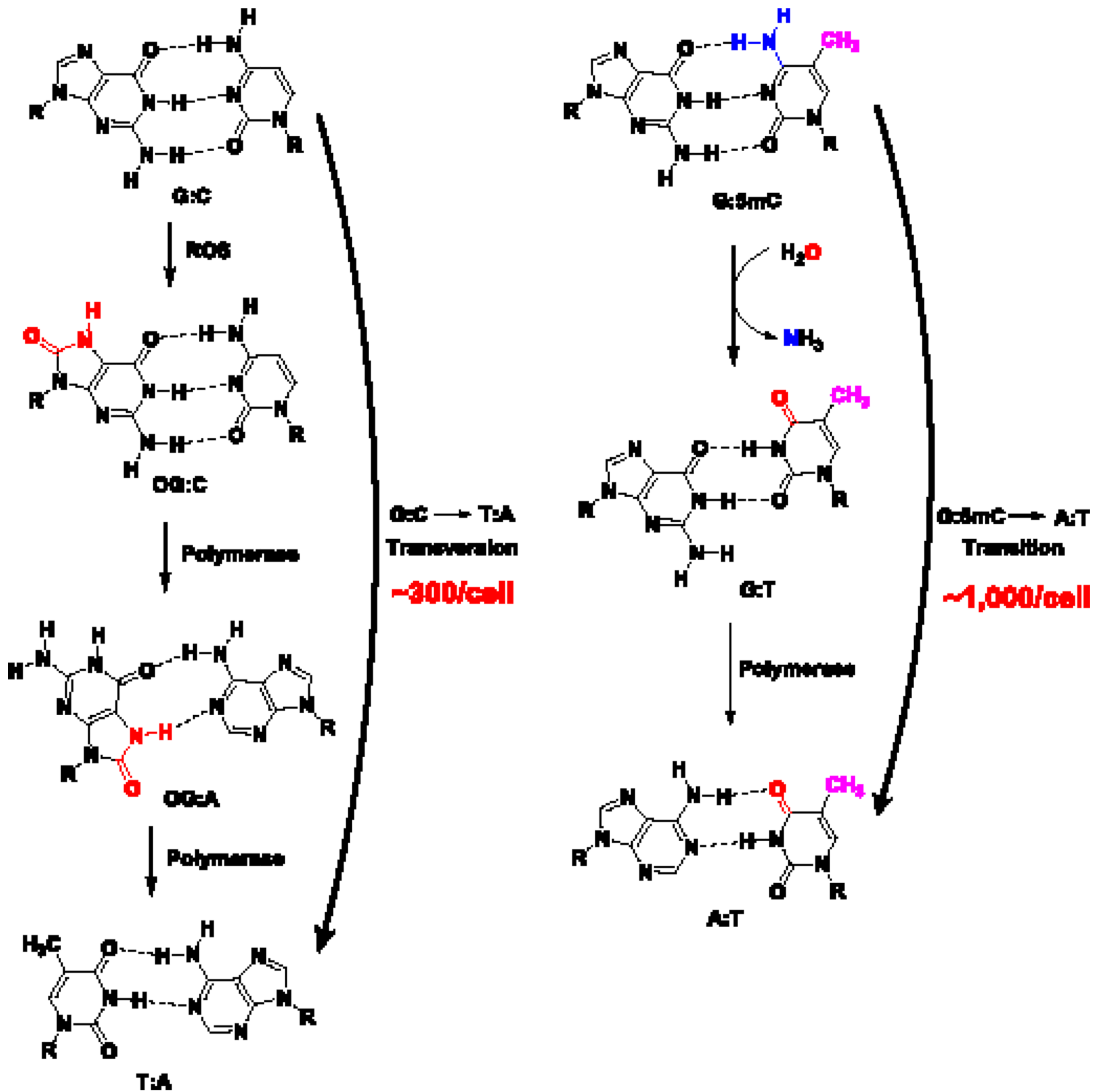

Fig. 4.
Pathways to generate genomic mutations for OG and 5mC.