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Regulation of DNA glycosylases and their role in limiting disease

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Abstract

This review will present a current understanding of mechanisms for the initiation of base excision repair (BER) of oxidatively-induced DNA damage and the biological consequences of deficiencies in these enzymes in mouse model systems and human populations.

Keywords

OGG1; *NEIL1,2,3*; *NTH1*; *glycosylase-deficient mice*; *metabolic disease*; *MUTYH*

Introduction

This review will present a current understanding of mechanisms for the initiation of base excision repair (BER) of oxidatively-induced DNA damage and the biological consequences of deficiencies in these enzymes in mouse model systems and human populations. In this review, we focus mainly on the DNA repair glycosylases important for the excision of oxidative lesions. The roles of other BER enzymes in the maintenance of genomic stability have been reviewed elsewhere [114,157]. Our discussion is designed to integrate with other reviews in this issue, specifically those by Jaruga and Dizdaroglu [112] on the comprehensive analyses of the formation and repair of oxidatively-damaged bases and by Delaney et al. [46] that examines the structural and biophysical consequences of these base alterations, as well as the mutagenic potential and the biological implications of oxidative DNA damage in repeating DNA sequences.

Initiation of base excision repair by DNA glycosylases

Although much of the focus of BER centres on the differential substrate specificities and kinetics of the DNA glycosylases, there is a cascade of pre-catalytic steps that must occur prior to enzyme-mediated catalysis: (1) non-specific DNA binding that facilitates the efficiency of locating sites of damage (scanning or one-dimensional, random diffusion on DNA), (2) specific DNA binding associated with partial nucleotide extrusion, (3) nucleotide

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flipping and stabilization of a bound enzyme-DNA complex and (4) enzyme-mediated catalysis.

Interactions with undamaged (non-target) DNA

Even in the absence of exposure to ionizing radiation, ultraviolet irradiation or exogenous chemical agents, cellular DNA sustains a variety of DNA lesions that include saturated pyrimidines, oxidized and fragmented purines, and base deamination and loss. Thousands of these lesions are formed in each cell per day, and if unrepaired, promote genomic instability. Considering that the human genome contains ~6.4×10^9 nucleotides per cell, the challenge for DNA glycosylases is to find and initiate repair at sites that occur at a frequency of one in several million undamaged nucleotides. This problem is not limited to DNA repair systems, but applies to rapid transcriptional regulation in which it was recognized that the kinetics of activator binding could not be rationalized by random 3-dimensional diffusion within a cell [18,184]. In this regard, the laboratory of Dr. Peter von Hippel led both the experimental and theoretical/computational approaches to this problem and proposed that specific DNA target location was possible by a directionally unbiased facilitated diffusion along the DNA modulated by electrostatic interactions [17,228]. However, until recently, the experimental data supporting these models were not sufficiently robust to distinguish between a sliding model that implies continuous contact between the protein and DNA and a microscopic hopping model that consists of a large number of association/dissociation events. In the latter model, multiple enzyme-DNA encounters could occur in which the protein does not escape the DNA strand to which it was originally bound, but at some low frequency, a dissociation event could result in transfer to another DNA molecule, effectively restarting the scanning process. It was also possible, especially for dimeric (or higher order) proteins, that direct inter-DNA (intersegment) transfer could account for translocation between strands.

When considering DNA glycosylases, facilitated diffusion has been demonstrated for T4-pyrimidine dimer glycosylase (T4-pdg) [54,55,69,78,79,137,138,158,159,162,191], Chlorella virus (cv)-pdg [71,72,148,191], *Micrococcus luteus* UV endonuclease [87], formamidopyrimidine glycosylase (Fpg or MutM) [57,61,62,202], uracil DNA glycosylase (UDG) from *Escherichia coli*, human and vaccinia virus [16,25,63,94,175], *E. coli* endonuclease VIII (Nei) [57], *E. coli* adenine DNA glycosylase (MutY) [59,60], *E. coli* endonuclease III (Nth) [57], 8-oxoguanine DNA glycosylase OGG1 [15,21,35,202] and human alkyl adenine glycosylase (AAG) [93]. For many of these investigations, a facilitated diffusion along DNA was inferred from experimental designs that measured clustered incisions in oligodeoxynucleotides containing two or more lesions in the same or complementary strands that were separated by varying distances. Alternatively, plasmid-based assays were designed in which limiting concentrations of the glycosylase were incubated with multiply-damaged supercoiled DNA under processive nicking conditions. Data from this assay revealed that in a subset of plasmids all lesions were incised, while other DNA molecules in the same bulk solution contained no nicks. The relative processivity of the facilitated diffusion nicking can be modulated by the ionic strength of the solution in which at low salt concentrations (generally < 50 mM), maximal processive nicking was observed, while at salt concentrations > 100 mM, the incisions were more random and not

clustered. These data strongly suggested that a major force mediating these interactions arose from electrostatic interactions between basic amino acid side chains (lysine, arginine and histidine residues) in the enzyme and the negatively charged DNA. Consistent with this hypothesis, site-directed mutagenesis was used to neutralize basic residues within many of the glycosylases, with concomitant decreases in clustered processive nicking activities [54–56,137,158–160,183].

Since the intracellular ionic strength is known to be in excess of 100 mM, the observations that *in vitro* processivity greatly decreases at physiologically relevant concentrations have led to speculation that this highly processive characteristic may not be germane in the context within the cell. In this regard, only a limited number of glycosylases have been studied. The intracellular processive nicking activity of a DNA glycosylase was first shown for T4-Pdg. Analyses of the *in vivo* kinetics of repair of UV-irradiated plasmid DNA was dose-dependent and subsets of plasmid DNAs became fully repaired prior to any cyclobutane pyrimidine dimers (CPDs) being removed from other plasmids [79]. The ability of the T4-Pdg processive nicking activity to confer cellular resistance to UV has been extensively demonstrated [54,56,158–160].

Although the observations described above suggest that a facilitated target search is ubiquitous for DNA glycosylases, most of these data do not address the actual mechanism that is used during the search. However, there are now several investigations that provide direct insight into this mechanistic issue [21,57,175]. Using single molecule imaging of tethered flow-stretched bacteriophage λ DNA, human OGG1 was shown to slide with persistent contact with DNA with a diffusion constant of ~5×10^6 bp^2/second, a value approaching barrierless Brownian motion [21]. This rate was virtually unaffected by varying the salt concentration between 10 and 100 mM, suggesting that hopping was not the operative mechanism. Further, as anticipated for this type of search mechanism, there was no directionality to the sliding. Given an average lifetime of binding of 0.025 seconds, this would calculate to an average sliding length of ~ 450 bp. However, the diffusion constant of hOGG1 was strongly pH dependent between pH 6.6 and 7.8, and this finding could be attributed to a positively charged His270 at the lower pH. This investigation also examined *E. coli* Fpg and determined that the rate of sliding was ~3.5×10^5 bp^2/second, an ~10-fold reduction relative to hOGG1; however, the authors noted that since the *E. coli* genome is ~3 orders of magnitude less complex than the human genome, this may compensate for the differential diffusion rates.

More recently, rotational diffusion rates on DNA were measured using quantum dot-labelled Fpg, Nth and Nei on YOYO-1 labelled λ DNA [57]. The results of this investigation were very consistent with that previously reported, for hOGG1, but diffusion rates segregated between either a slow, sub-diffusion rate (< 0.01μm^2/sec) or a fast, near diffusion limited rate (> 0.1μm^2/sec).

In contrast to these data, Porecha and Stivers [175] demonstrated that *E. coli* UDG primarily uses a 3-dimensional search through bulk solution, moving between DNA molecules by closely spaced hopping, coupled with a very short sliding distance of ~10 bp. This short range sliding is consistent with UDG trapping transiently extrahelical uracil residues.

Extrapolation of these data to human UDG suggests that this would be a highly efficient mechanism for monitoring the nuclear DNA.

DNA bending and nucleotide flipping

Having established that DNA glycosylases generally reduce the dimensionality of the search for damaged bases, these data did not address how these enzymes flip nucleotides by ~180° to an extrahelical position within the active site pocket. To achieve this state, the remaining hydrogen bonds of base-pairing must be broken, base stacking forces must be disrupted and the sugar phosphate backbone must assume strained conformations. For the majority of glycosylases, it is the damaged (or mismatched) nucleotide that is moved to the extrahelical position; however, the T4-Pdg and presumably other closely related UV-specific glycosylase-abasic (AP) site lyases, flip the undamaged purine that is opposite to the 5′ pyrimidine of the CPD [74].

The requirement for positioning a nucleotide in an extrahelical position overcomes steric hindrances to the C1′-N glycosidic bond in which activated water or nucleophilic displacement at the C1′ position can occur [96,205]. Further, this reaction requires accessibility to hydrogen bond acceptors on the base so that the negative charge formed in the Michaelis complex can be neutralized by the enzyme. Thus, given the necessity for this large structural transition, what are the internal dynamic motions on DNA that allow this rapid sampling and how might glycosylases increase sampling discrimination of target versus non-target (or normal) DNA?

Specific binding represents the extremely rare event in which the vast majority of nucleotides were rejected through a series of increasingly stringent thresholds. As described above, the native structure of non-damaged duplex DNA is energetically stabilized through hydrogen bonding and base stacking, and depending on local sequence context, it undergoes breathing or motions mimicking the trajectory of an extrahelical base. Computational analyses of the reaction coordinate for spontaneous nucleotide flipping reveals large increases in the free energy barrier during the initial breaking of hydrogen bonds that occurs in the first ~40° of the 180° full rotation [12,13,103]. For further rotation beyond achieving the partial solvent exposure, no additional constraints to full extrahelical extrusion are anticipated. NMR spectroscopic analyses of imino proton exchange suggest an ~100-fold differential in the open/closed equilibrium comparing G/C versus A/T base pairs [82,83,133]. Further, computational investigations of flipping at non-damaged base pairs suggest that the purine is far more likely to be extruded than the complementary pyrimidine, with G > 100-fold over C and A ~6-fold over T [205].

Given these parameters in non-damaged DNAs, it would be anticipated that damaged or mismatched sites would be more susceptible to spontaneous opening. The Stiver's group directly tested this hypothesis by creating artificial base pairs with 1, 2 or 3 hydrogen bonds between T (or U) with nebularine, adenine or diaminopurine and measured UDG to these DNAs [205]. These data revealed a linear correlation between the dissociation constant and the equilibrium constant for base pair openings. Overall, the conclusions from this study strongly suggest that glycosylases may take advantage of perturbations in base pair stability to achieve a final bound complex. Similar adduct-induced destabilization of helix parameters

have been measured for thymine glycols (Tg) [121], formamidopyrimidine (Fapy)-dG [88,140], Fapy-dA [88] and 8-oxoguanine (8-OH-G) [204].

Given the inherent and damage-induced internal motions of nucleotides within DNA, as glycosylases rotationally diffuse on DNA, they appear to probe the stability of the DNA helix through protein-induced pinching of the DNA to expand the minor groove [28,99,129,130,170]. Additionally, amino acid wedges, as illustrated by *E. coli* Fpg, appear to facilitate the transient helix opening [57,66,202]. As mentioned above, using quantum dot-labelled Fpg, Dunn et al. [57] demonstrated that there were at least two modes of scanning which could be distinguished by F114 probing the stability of the DNA. This conclusion was inferred since a F114A mutant only showed the fast diffusion mode. These data are consistent with previous mutagenesis analyses of Fpg [202]. Detailed analyses of the structural determinants of these bindings have been reviewed [225].

hOGG1 as a model for sequential interrogation of damaged DNA

In order to illustrate many of these pre-catalytic steps, the following section will highlight data for human OGG1, primarily from the Verdine laboratory.

In order to assay glycosylase-induced changes in non-damaged DNA, Chen et al. [35] used atomic-force microscopy (AFM) of hOGG1 bound to a fully duplex 1234 bp DNA fragment, with initial interactions likely driven by electrostatic interactions. Somewhat unexpectedly, the majority (~2/3) of bound DNAs contained a sharp 70° bend that was not detected in the samples without enzyme. The remaining molecules contained a bend of ~10°. These data were compared to bend angles induced by hOGG1 following binding to either a 1024 or 1349 bp fragment containing a single site-specific 8-OH-G in which specific and non-specific DNA binding could be distinguished based on distance from an end. This bend angle averaged ~71°. Given the close similarity of the specific and non-specific bend angles, the interpretation of these data was that the non-damaged DNA-hOGG1 complex likely represents a structure containing an extrahelical nucleotide, since the enzyme-DNA contacts required for binding an extrahelical 8-OH-G would be comparable to those for non-damaged DNA. Comparable studies with AlkA also revealed a bend angle of 72° on non-damaged DNA [35], a value that corresponds well with the bend in the co-crystal structure of AlkA bound to DNA containing a 1-azaribose abasic nucleotide [98]. These data suggest that enzyme-induced bending of non-damaged DNA facilitates nucleotide flipping as part of the target search.

These AFM data also suggested that it may be possible to trap non-productive nucleotide flipping of non-damaged DNA. To address this question, hOGG1 was engineered to be both catalytically inactive (K249Q) and capable of crosslinking (N149C) to the estranged cytosine of an 8-OH-G:C pair [15]. The co-crystal complex revealed a structure that was nearly identical to the reduced catalytic DNA-OGG1 complex [28]. Given the feasibility of the crosslinking strategy, hOGG1 N149C was co-crystallized with undamaged DNA in which the 8-OH-G:C was substituted for a normal G:C base pair. This structure revealed that the DNA-hOGG1 complex was severely bent to 80°. However, in contrast to the full insertion of the 8-OH-G into the catalytic site of the enzyme [28], the non-damaged G was excluded from this pocket and occupied an exo-site ~5° outside the pocket [15]. Although

the exo-site bound G interacted with two of the active site residues (Phe319 and His270), these contacts were significantly different than that observed with the bound 8-OH-G, thus effectively excluding the undamaged base from the active site. The authors conclude that binding in the exo-site is a late intermediate in the overall base flipping pathway, and that perturbations in the sugar phosphate backbone 3′ to the lesion may represent early (pre-hydrogen bond breaking) probing of the structure.

Overall the detailed biophysical and biochemical dissection of the sequentially restrictive motions in both the DNA and hOGG1 provide elegant insight into the precision by which 8-OH-G lesions can be located and excised from the genome, without aberrantly initiating repair processes at non-damaged DNA sites. An equally compelling series of experiments have also been published for the Fpg (MutM) glycosylase/AP lyase [14,57,65,66,177] and endonuclease III [67].

Enzyme-mediated catalysis

In the original publications for many of the glycosylases with an associated AP lyase activity, investigators were unaware of the underlying chemical mechanisms responsible for cleavage of the phosphodiester bond and thus, the term `endonucleases' was and, in some cases, is still used to name these enzymes (T4 endonuclease V, endonuclease III, endonuclease VIII, etc.). Pioneering work by Bailly and Verly [10,11], established that the DNA incision products were inconsistent with endonucleolytic cleavage mechanisms, but rather these enzymes were glycosylases with associated AP lyase activities. In this class of enzymes, the T4-Pdg was the first glycosylase/AP lyase to have its active site nucleophile identified [198]. It had been hypothesized that a primary amine (probably an ε-amino group of lysine) would catalyse a nucleophile attack on the C1′ of the deoxyribose, generating a Schiff base intermediate that could be trapped by concomitant reduction with $NaBH_4$ or $NaCNBH_3$. The reduced imine intermediate was stable and amenable to a variety of mapping and structural studies. However, mapping the active site residue revealed that the N-terminal threonine was covalently linked to the DNA through $NaBH_4$ reduction. These data suggested that the α-amino group of the N-terminus was necessary for glycosylase bond cleavage. Subsequent site directed mutagenesis and X-ray crystallography confirmed these conclusions [50–52,74,145,147,149,198,199]. The knowledge that the chemistry of all the glycosylase/AP lyases proceeded through a common covalent intermediate has been a key tool in the identification of active site residues and solving covalently-linked co-crystal structures [29,148,174,208]. To date, the co-crystal structures of stably-trapped covalent catalytic intermediates have been solved for the following glycosylase-abasic site lyases: endonuclease III [67], endonuclease VIII [239], OGG1 [64], Fpg [66,73] and T4-Pdg [74]. These structures unequivocally establish the active site nucleophile for each of these enzymes. In addition, given that these structures represent post-glycosyl bond scission, but pre-β or pre-β/δ elimination structures, comprehensive analyses of these structures provides the framework on which to construct plausible models of catalysis. Such models have received strong support based on biochemical analyses of site-directed mutagenesis, coupled with phylogenetic conservation of presumed active site residues. Since the identities of these active site residues are enzyme specific, the reader is referred to the primary literature for

hypotheses concerning the roles of individual side-chains in the concerted sequential incision reactions.

Regulation of DNA glycosylases involved in oxidative DNA damage repair

While biophysical and biochemical analyses yield fundamental insights into the pre-catalytic and catalytic activities of these DNA glycosylases, one of the limitations of such investigations is that the vast majority of these studies have been performed using recombinant enzymes expressed in *E. coli*, many of which are not of full length due to inherent expression and/or purification difficulties. Additionally, many glycosylases have multiple splice variants, and post-translational modifications that are not accounted for in studies using recombinant proteins. Thus, extrapolation of the biochemical data to cellular studies must take these limitations into account.

The regulation of the activities of DNA glycosylases is both temporally and spatially important to maintain genomic homeostasis within a cell, tissue and organism. The importance of this regulation is evident in the fact that cells have multiple levels of control to dynamically modulate the BER system. Therefore, it is interesting to review how differential transcriptional control, intracellular trafficking and post-translational modification can affect the overall role of these enzymes in the context of intact organisms. Although crosstalk between repair and response pathways, as well as protein-protein interactions within pathways, are important in the overall function of BER, these are beyond the scope of this review.

Regulation of gene expression

Promoter attributes

Evidence indicating that there are complex mechanisms governing the regulation of DNA repair enzymes, suggests that specific DNA lesions or mismatches may be repaired by different mechanisms and/or efficiencies, depending on the phase of the cell cycle, the cell type or its subcellular localization. In addition, cellular responses to external stimuli, that is, a damage response pathway, may result in regulation of either the levels of repair proteins and/or their localization within the cell. Much of this regulation may occur at the transcriptional level; however, with the exception of the *Ogg1* gene, not much is known about the promoter structures or enhancer/repressor elements for the other oxidative damage-specific glycosylases.

The *hOgg1* promoter region was cloned and sequenced and reported not to contain TATA or CAAT boxes, suggesting that it serves as a housekeeping gene [47]. However, analyses of the *Ogg1* promoter have revealed potential transcription factor (TF) responsive elements including two CpG islands, an Alu repeat, Nrf2, E2F, SP1 and NF-YA TF binding sites [47,132,142]. In addition, two inverted CCAAT box motifs have been identified within the − 121 to − 61 region, that are specific binding sites for the NF-YA transcription factor. Mutations at these sites abolished *Ogg1* promoter activity, suggesting an important role for NF-YA in the regulation of *Ogg1* expression [86]. Cadmium suppressed Sp1 TF binding to the *Ogg1* promoter and subsequently down-regulated *hOgg1* expression [236]. Interestingly,

the transcriptional activity of the *Ogg1* promoter was reduced in kidney cells and tissues deficient in tuberin, the protein encoded by *tsc2*, a gene deficient in patients with tuberous sclerosis disease. Tuberin regulation of *Ogg1* expression appears to be *via* the AP4 TF that was shown to bind to the 3′ end of the *Ogg1* promoter [85]. An additional transcriptional control mechanism for OGG1 has been proposed that involves the tumour suppressor gene, Brca1. Elevated levels of *Brca1* resulted in increased expression of both *Ogg1* and *Nth1* [192], and it was suggested that Brca1 acts as a co-activator of the OCT-1 TF (octamer-binding transcription factor) [192,193]. Interestingly, *Oct1*$^{-/-}$ fibroblasts are more sensitive to oxidative stress and exhibit low levels of *Nth1* promoter activity [192].

The *Nth1* promoter has also been cloned and sequenced. While it does not contain a TATA or CAAT box [109], it does contain a CpG island having several putative TF binding sites [108,109]. Characterization of the genomic structure of *Nth1* has also revealed multiple transcription initiation sites [109]. The *Nth1* gene lies immediately adjacent to the *tsc2* gene [109]. Although the transcription of *tsc2* and *Nth1* are bidirectional, both genes are regulated by a common minimal promoter that contains two ETS binding sites (EBS) [100,108]. The promoter activity of *Nth1* is suppressed by ELF-1 TF binding to the EBS [100]. Both *Nth1* and *Elf-1* expression are down-regulated in rat liver during acute hepatitis in the Long–Evans Cinnamon (LEC) model system for Wilson's disease [194]. The authors attribute this down-regulation to increased protein oxidation that affects binding of TFs to the EBS, thus decreasing promoter activity and potentially contributing to carcinogenesis.

Cell cycle-dependent expression

Two BER glycosylases, MUTYH and NEIL1, appear to have a replication-associated function, and accordingly, the genes encoding these enzymes are up-regulated during S-phase of the cell cycle. Expression levels of *Mutyh* increase and MUTYH protein accumulates in the nucleus during S-phase and co-localizes with PCNA at replication foci [23]. It has also been demonstrated using an *in vivo* repair system that DNA replication enhances the repair of 8-OH-G:A mispairs and that a significant part of this replication-associated repair depends on MUTYH [90].

NEIL1 mRNA and protein levels were shown to increase in S-phase, suggesting a potential role in replication-coupled repair [91]. The biological significance of this observation may be correlated with the increased catalytic efficiency of NEIL1 for lesions in bubble structures [53]. In contrast, *Neil2* gene expression did not vary with cell cycle [92].

hNth1 has been shown to have cell cycle dependent expression [24], with increased expression during early and mid-S phase of the cell cycle [141]. In addition, while OGG1 activity in irradiated cells did not vary during the cell cycle, *Nth1* was expressed at higher levels in G1 relative to G2 phase [33]. The biological significance of these changes has not been demonstrated.

Differing reports have been published concerning the cell cycle dependent expression of *Ogg1*. Data in reports supporting a lack of cell cycle control include no change in: OGG1 protein levels, DNA glycosylase activities, mRNA levels based on ribonuclease protection assays or Ogg1-luciferase expression reporter assays [23,24,33,47]. However, using a GFP-

hOgg1 fusion, Luna et al. [142] showed cell cycle dependent expression of nuclear and mitochondrial forms of OGG1. The implications or general applicability of these findings at an organismal level have not been established. However, as mentioned previously, the *Ogg1* promoter contains NF-Y and putative E2F binding sites, both of which are suggestive of cell cycle dependent expression [142]. Thus, for many of these glycosylases, there is a need to determine the significance of these differential cell cycle dependent expression patterns.

Tissue-specific expression

In addition to transcriptional regulation, tissue distribution appears to be an additional level of control in the regulation of the BER glycosylases. NEIL1 is reported to have broad tissue distribution, but individual laboratories have noted significant organ-specific differences. Based on Northern analyses of adult human tissue, *Neil1* mRNAs are present at the highest levels in liver, thymus and pancreas [91,153]. *In situ* hybridization and Northern blot studies have also demonstrated that mouse *Neil1*, *Neil2*, *Ogg1* and *Nth1* are all ubiquitously expressed in the brain and the expression of *Neil1* increased with age [185]. In contrast to *Neil1*, *Neil2* had highest levels of expression in testes and skeletal muscle [92]. Mouse *Neil3* had an expression pattern different than the other glycosylases examined, being selectively expressed in areas of the brain containing stem cells, suggesting that *Neil3* may be important for stem cell differentiation [185].

hOgg1 is expressed at highest levels in the thymus, testis, intestine, brain and germinal centre of B cells [124,161,182]. Similar to *Neil1, Ogg1* shows an increase in expression with age in the rodent brain [185]. Specific analyses of *Ogg1* transcripts in the rat CNS showed widespread and heterogeneous expression in the different brain regions [226]. Using nuclear and mitochondrial extracts from various mouse tissues, the activity of mOGG1 was shown to be lower in the mitochondria, as compared to the nucleus, and the highest activity was observed in the testis, consistent with the high expression levels observed in this tissue [116].

Interestingly, differential expression of *Ogg1* was demonstrated in different cell populations in peripheral tissues [226]. Similar to these findings, the nuclear form of OGG1 was differentially expressed in skin, with the highest levels found in the upper granular layer of the epidermis and little to no expression in the middle and basal compartments [113]. It was demonstrated that this difference in expression was not due to cell-type but rather due to the differentiation status of the cells in the various skin layers. Differentiated keratinocytes in the upper layers were shown to have more OGG1 expression than non-differentiated keratinocytes in the lower layers [113]. Additionally, *Ogg1* knockout mice are significantly more sensitive to developing UV-induced skin tumours than wild-type or heterozygous mice [122], and this sensitivity is associated with pro-inflammatory responses [123].

Nth1 is ubiquitously expressed, but some tissues such as heart and brain show higher levels of expression and differing levels of activity [109,116]. In rodent brains, *Nth1* was constitutively expressed throughout the lifespan of the animals [185].

Stress-induced regulation

There are several examples in the literature of BER enzymes being regulated as a function of exposure to oxidative stress. *Ogg1* mRNAs and protein levels have been shown to increase in response to various insults, including H_2O_2, methyl methanesulfonate (MMS), arsenite, asbestos, diesel exhaust particles, cigarette smoke and diethylmaleate [31,44,117,125,200,219]. The increased expression observed following MMS treatment was shown to be dependent on regulation of the NF-YA TF binding site [132]. In addition, several studies have noted that *Ogg1* is upregulated following focal ischemia in rodent brains [126,135]. In contrast to the stimulation of *Ogg1* expression, sodium dichromate decreased *Ogg1* in human lung epithelial cells [97], and cadmium decreased *hOgg1* expression in rat lung and alveolar cells [176]. Cadmium down-regulation of *Ogg1* expression was shown to be *via* suppression of Sp1 binding to the *Ogg1* promoter [236].

Protein levels of MUTYH are up-regulated in response to $CoCl_2$ -induced hypoxia and chronic H_2O_2 exposure [80,229]. In addition, transcriptome analyses of tissue from rats fed quercetin showed an induction of *Mutyh* expression [48,81]. Levels of *Neil1* mRNA have been shown to be elevated following ROS [43] and aniline treatments [143].

While many of these treatments induce production of free radicals, no transcriptional or post-translational induction by low-dose IR (0.5–2.0 GY) was observed for *Nth1*, *Ogg1* or *Neil1 - 3* [110]. Likewise, no change in mRNA levels was observed for *Ogg1*, *Neil1* and *Neil2* in rat hippocampal cultures exposed to oxygen and glucose deprivation [186].

The expression and activities of OGG1 are also regulated as a function of both aging and exercise. Age-associated impairment of OGG1 cellular localization has been reported. Specifically, for the OGG1β (mitochondrial form), a large amount remains in the precursor form localized in the outer membrane and intermembrane space [210,211]. In addition, hepatic OGG1 mitochondrial activity has been reported to increase during aging, while the nuclear activity decreases or remains the same [45]. In contrast, an age-associated decrease in hOGG1 activity was demonstrated in human peripheral blood lymphocytes [36]. Also, studies in brain cortical mitochondria have revealed a robust upregulation of *Nth1*, *Ogg1* and *Neil1* in middle-age, followed by a significant drop in older animals [77]. This pattern of regulation was reported to be specific to the cortex, as age-associated changes were not observed in hippocampal mitochondria [77]. In contrast, NTH1 and OGG1 activities were increased in cerebellar mitochondria in older animals, indicating both tissue-specific and age-associated regulation of these enzymes. It will be interesting to determine if oxidative stress load and repair capacity are correlated with the relative regulation of these enzymes.

Expression levels and intracellular accumulation and distribution of OGG1 have been examined in exercise trained and detrained (rested) rat skeletal muscle [180,181]. These data have revealed that red skeletal muscle had increased nuclear OGG1 activity as compared with white skeletal muscle and that exercise training further increased OGG1 activity in red muscles, but decreased activity in white muscles. These studies also showed a surprising reciprocal relationship of mitochondrial OGG1 activity in red muscle, whereby exercise training decreased OGG1 activation in mitochondrial lysates, whereas detraining increased this activity [180]. The physiological relevance of this paradoxical regulation of OGG1 in

oxidative muscle types, which rely heavily on aerobic mitochondrial metabolism, is as yet unclear.

Post-translational modifications

Post-translational modifications to DNA glycosylases may alter or regulate subcellular localization, substrate binding and protein–protein interactions, and may include acetylation, phosphorylation, sumoylation and ubiquitination [2,223]. Acetylation of hOGG1 at Lys338 and Lys341 increases glycosylase activity by increasing enzyme turnover through a reduction in the enzyme's affinity for AP site products [20]. hNEIL2 has also been shown to be acetylated (Lys49 and Lys153), with modification at Lys49 inactivating both the glycosylase and lyase activities [19]. The oxidation of OGG1 following cadmium exposure has also been reported [26], and the decreased activity of the Ser326Cys OGG1 variant is associated with the oxidation of the Cys326 residue [27].

Intracellular localization of OGG1 may be regulated by phosphorylation via PKC [42,102,142]. In addition, OGG1 has been shown to interact with, and be phosphorylated *in vitro* by both the c-ABL and CDK4 kinases [102]. However, only phosphorylation by CDK4 altered the glycosylase activity of the OGG1 enzyme.

While it had been previously suggested that MUTYH can be phosphorylated *in vitro* [81], Parker et al., demonstrated *in vitro* phosphorylation of MUTYH by PKC and this phosphorylation appeared to stimulate the glycosylase activity of the enzyme [172]. Impaired phosphorylation of MUTYH was correlated with defective 8-OH-G: A repair in colorectal cancer cell lines with wild-type alleles of the gene [172]. Further, in a recent study using baculovirus-expressed recombinant MUTYH, mass spectrometry analyses identified Ser524 as a phosphorylation site on MUTYH [120]. Interestingly, this residue is located within the PCNA binding domain of the glycosylase, and the authors speculate that the phosphorylation may contribute to the regulation of the replication-coupling of the repair activity via PCNA binding.

Subcellular localization

Nuclear and mitochondrial translocation is another form of regulation of DNA repair activities within cells. It appears that the subcellular localization of many DNA glycosylases is regulated via alternate RNA transcripts (generated either through splicing or alternate start sites) that contain a localization signal. Consensus and functional nuclear localization sequences have been identified for NTH1, NEIL1, NEIL3, MUTYH and OGG1 [119]. The NTH1, MUTYH and OGG1 proteins contain classical nuclear localization sequences (NLS) (mono and bi-partite basic amino acid clusters) and are presumably imported into the nucleus by the importin α/β pathway. While NEIL2 does not contain a classic NLS sequence, it also primarily localizes to the nucleus [91].

hOgg1 has seven splice variants that have been classified into two types based on the last exon present [161]. The major expressed forms are *Ogg1*α (Type 1a) and *Ogg1*β (Type 2a). Ogg1α contains a nuclear localization sequence at the C-terminal end but has been found in both mitochondria and nuclei [45,212]. In contrast, the product of the Ogg1β gene is

exclusively mitochondrial [161,212]. OGG1β has been reported to be catalytically inactive in the repair of 8-OH-G damage in mitochondrial DNA [89], but this form may affect apoptotic responses [166]. Thus, the *Ogg1*α splice variant may account for most of the cell's activity against 8-OH-G.

A variety of cell types and experimental protocols have been used to track the intracellular distribution of OGG1 as a function of phase of the cell cycle or following damage induction. A GFP-fused hOGG1 was shown to co-localize to condensed chromosomes during mitosis and associate with chromatin and the nuclear matrix during interphase [42]. In addition, mOGG1 and mNEIL2 were shown to associate with microtubules during interphase and spindle assembly during mitosis [38,39]. During S phase, hOGG1 was found to be predominantly in the nucleoli [142]. However, following laser-induced oxidative damage in *Ogg1*$^{-/-}$ MEFs, GFP-tagged OGG1, which is accumulated in discrete nuclear regions, re-localizes rapidly (within 2 minutes) to foci [240].

Additionally, using a stem-loop oligodeoxynucleotide containing an 8-OH-G lesion and terminal fluorescent labels as `molecular beacons' for mOGG1 activity *in situ*, Mirbahai et al. [152] demonstrated that OGG1 activity was enhanced following potassium bromate-induced oxidative stress. Surprisingly, the activity was exclusively found in the mitochondria, but this increase was not due to transcriptional activation of the *Ogg1* gene.

The Ser326Cys variant of OGG1, has been shown to have impaired localization that is likely due to altered regulation via phosphorylation at Ser326 [142]. The mutant protein was shown to be imported into the nucleus, but excluded from the nucleoli. Associations with the soluble chromatin, nuclear matrix and condensed chromatin are also disrupted in the Cys326 variant [142].

Recruitment of glycosylases to damage foci

Although in previous sections, the mechanisms by which DNA glycosylases initially locate to and form precatalytic complexes at sites of specific DNA lesions have been discussed, this represents only the first step in assembling the full complement of BER enzymes that are required to complete repair. To experimentally address the subsequent assembly and catalytic processing steps, several studies have examined the kinetics and/or localization of BER glycosylases at damage-specific sites within the nuclei, using laser and opaque filter systems to create discrete areas of damage within the nuclei.

In an effort to understand the kinetics of BER protein assembly at sites of DNA damage, Lan et al. utilized *in situ* analyses of GFP-tagged glycosylases, NTH1, OGG1, NEIL1 and NEIL2 [127]. These data demonstrated that the glycosylases accumulated at sites of irradiation in mammalian cells with maximum accumulation at 30 minutes post-irradiation by a 365 nm pulse laser with F25 filter (predominantly inducing substrates for NTH1 and NEIL1) [127].

In additional studies, OGG1 was shown to be recruited to the nuclear matrix and co-localized to `nuclear speckles' following UVA irradiation [30]. On further examination, the Radicella group demonstrated that OGG1 was excluded from heterochromatin and recruited

to euchromatin following potassium bromate-induced oxidative stress [3]. In both reports, downstream BER enzymes were also recruited to these foci. Consistent with previous reports, the OGG1-GFP protein was exclusively nuclear in non-treated cells, and the association with the chromatin fraction was independent of the cell cycle.

Using immunofluorescence, it was demonstrated that MUTYH and polymerase λ are recruited to DNA damage induced by UVA laser microirradiation [222]. Both proteins were also significantly up-regulated in HeLa cells following H_2O_2 exposure. Using whole cell extracts from HeLa cells and a reversible crosslinking protocol on an A:8-OH-G containing substrate, it was shown that not only were MUTYH and polymerase λ recruited to sites of ROS-induced damage, but also PCNA, FEN1 and DNA ligases I and III. Overall these data suggest that BER foci are assembled at the sites of these mispairs [222].

Intracellular localization of NEIL1 has been shown to be primarily nuclear, based on data demonstrating NEIL1 activity in nuclear extracts and using recombinantly-tagged NEIL1 constructs [153,201,213,214]. NEIL1 associates with condensed chromosome and centrosomes [95]. Transient transfections of GFP-fused NEIL1 revealed intracellular sorting to the nucleus with accumulation in the nucleoli, resembling OGG1 [42,142,153]. It should be noted that most of the above studies used only one of the primary transcripts for *Neil1*. If this transcript encodes the nuclear targeted form of the enzyme, then alternatively spliced transcripts could direct the enzyme to other intracellular locations. This is particularly germane since the laboratory of Dr. Vilhelm Bohr (NIA) demonstrated mitochondrial localization of NEIL1 by both Western blot analyses and activity assays [101]. These data provide evidence that NEIL1 may be involved in mtDNA repair. A mitochondrial role for NEIL1 was further supported by observations of increased levels of PCR-blocking lesions and deletions in mtDNA [224] and reduced hepatic mtDNA content and mitochondrial protein levels following oxidative stress in *Neil1*$^{-/-}$ mice [197,224].

Outside the putative catalytic domain, MUTYH has both N-terminal and C-terminal domains involved in subcellular localization and protein-protein interactions. The MUTYH sequence contains both an NLS and a putative mitochondrial targeting signal, and the enzyme exists in multiple forms in both the mitochondria and the nucleus [164,212,215,218]. It is presumed that deficiencies in the nuclear form are responsible for the tumour suppressor mutations seen in MUTYH-associated polyposis patients.

In addition to mitochondrial localization, there is evidence that the mitochondrial forms of OGG1 and MUTYH are associated with the inner membrane of the mitochondria [155,161,164,206]. These data are consistent with the fact that mtDNA is known to be partially associated with the inner membrane [1,156], the predominant site of cellular ROS production, through membrane interactions near the origin of replication. A recent report suggested that the association of mtDNA to the membrane was essential for base excision repair and repair-associated DNA synthesis [22].

MUTYH has a classical monopartite nuclear localization signal composed of a single cluster of basic amino acids, RKKPR [218]. It has been reported that there are three primary transcripts, α, β and γ, which generate more than nine different isoforms of MUTYH [165].

In fact, 10 isoforms could be identified in Jurkat cells by amplification of MUTYH cDNAs [164], but there was no evidence that all these cDNAs encode proteins within the cell. Takao et al. [212,215] have shown that an epitope-tagged full-length (type α) MUTYH in COS-7 cells is localized predominantly to the mitochondria, and that an alternative transcript was localized to the nucleus. Our lab and others have also identified various forms of the protein by Western analyses, both nuclear (52/53kD) and mitochondrial (57 kD) [23,164]. The mitochondrial localization of p57 was specific to the inner membrane, similar to that observed for the hOGG1 - 2a [161]. The nuclear form (type β) is translated from the second AUG and lacks the mitochondrial targeting signal. It appears for intracellular trafficking that the nuclear localization signal is not as dominant as the mitochondrial targeting signal, and the mitochondrial form appears to be the most prevalent in an asynchronous population of cells [23,212]. Three alternatively spliced forms of MUTYH have been identified in rodent cells. These three mRNAs encode two different isoforms of MUTYH protein (50 kDa and 47 kDa), both of which lack the mitochondrial targeting signal [104]. The rodent MUTYH is primarily localized to the nucleus.

Interestingly, haplotype variations of MUTYH that are predicted to generate Pro18Leu and Gly25Asp missense mutations, have been shown to alter the localization of MUTYH and be associated with an increased risk for colorectal [34] and gastric cancer in a Chinese population [238]. This mutation, which is in close proximity to the mitochondrial targeting signal, was associated with a shift from predominantly mitochondrial localization to a dual localization, both mitochondrial and nuclear for the mutant protein [34].

Several reports demonstrate both nuclear and mitochondrial localization of NTH1 [106,212]; however, GFP-tagged hNTH1 was exclusively localized to the nucleus [107,141]. Interestingly, human and mouse NTH1 are differentially sorted, with the hNTH1 predominantly localizing to the nucleus and the mNTH1 predominantly localized to the mitochondria [107]. Three different isoforms of NTH1 result from the use of multiple initiation sites for transcription; however, no difference in localization was noted for any of the isoforms, which were all exclusively nuclear [107]. Deletion of the NLS resulted in mitochondrial localization of NTH1 [107].

Glycosylases in disease models

While much is known about the biochemical roles of these DNA glycosylases, their physiological role in the development of chronic diseases including cancer is only beginning to be understood [232]. These advances are largely due to insights gained from animal models of glycosylase deficiencies and inactivating mutations reported in human cancers. At first glance, it appears that several mouse models of glycosylase deficiency have no overt phenotype, arguing for the existence of multiple back-up glycosylases. However, a closer look at some of the existing animal models has revealed as yet underappreciated roles for these DNA repair enzymes in modulating disease risk.

Carcinogenesis

As discussed above, 8-OH-G is one of the most commonly generated lesions in response to oxidative stress in both nuclear and mitochondrial DNA. Therefore, given the presumed importance of this lesion, a defect in OGG1, the main glycosylase responsible for repair of 8-OH-G would be expected to result in a severe pathogenesis associated with aberrant repair of oxidative lesions. In support of this reasoning, OGG1 deficiency has been shown to cause a mutator phenotype in both yeast and *E. coli*, resulting mainly in increased G to T transversions [150,217]. Additionally, several inactivating mutations of OGG1, with Ser326Cys being the most prevalent, have been described in human lung squamous cell carcinomas [37,131,139,167,173,207,231], orolaryngeal [58], esophageal [235], kidney [8,9,37] and gastric [60] cancers. However, no OGG1 mutations have been found to be associated with skin basal cell carcinomas [227].

Interestingly, in multiple independent *Ogg1*$^{-/-}$ mouse models either no effect or only a mild effect on carcinogenesis can be attributed to an OGG1 deficiency. Despite loss of activity against 8-OH-G lesions and a consequent age-related accumulation of 8-OH-G in nuclear DNA of liver [118,168], *Ogg1*$^{-/-}$ mice were shown not to develop more spontaneous tumours than wild-type controls [118]. Several pieces of experimental evidence point to the existence of a back-up repair system, including the observation that in a rapidly proliferating tissue such as testis, *Ogg1*$^{-/-}$ mice were not found to have an increase in 8-OH-G accumulation, and a slow repair capacity for 8-OH-G was seen in *Ogg1*$^{-/-}$ MEFs, [118]. It was hypothesized that this repair capacity, possibly transcription coupled NER, along with endogenous MUTYH activity helps maintain a low steady-state level of DNA damage in rapidly proliferating tissues, despite an OGG1 deficiency [118].

Independent studies using a novel *Ogg1*$^{-/-}$ mouse model have revealed a significant increase in 8-OH-G lesions in *Ogg1*$^{-/-}$ mice. This was accompanied by a significant increase in mutations in a transgenic glutamic-pyruvate transaminase (*gpt*) gene, but no increase in tumour formation was detected in this model [151]. Using the same strain of *Ogg1*$^{-/-}$ mice, subsequent studies have reported that treatment with potassium bromate, a known oxidative stress-inducing agent, resulted in dramatically higher accumulation of 8-OH-G in both liver [5] and kidney [6,7] of *Ogg1*$^{-/-}$ mice, without any associated increase in spontaneous tumour formation up to 60 weeks of age [5,7]. Furthermore, following a partial hepatectomy, OGG1-deficient livers showed the same capacity to regenerate as WT livers, despite a continued accumulation of 8-OH-G levels and G to T transversions, indicating the absence of any significant replication-coupled repair in *Ogg1*$^{-/-}$ mice [5]. No increase in tumorigenesis was reported either in kidneys or livers of these mice.

In contrast to these results, another group, using an independently derived *Ogg1*$^{-/-}$ mouse model, demonstrated an increase in spontaneous lung adenoma/carcinomas in *Ogg1*$^{-/-}$ animals at 78-weeks of age, correlating with an accumulation of 8-OH-G in their genomes [196]. In the same study, knocking out the *Mth1* GTPase in addition to *Ogg1* resulted in a further increase in 8-OH-G levels, but did not cause a commensurate increase in lung tumorigenesis [196], thereby seemingly dissociating 8-OH-G accumulation from carcinogenesis. This result is particularly puzzling considering that *Mth1* knockout mice

have been shown to have a greater incidence of lung, liver and stomach cancers compared to wild-type controls, as early as 18 weeks of age [220].

Apart from OGG1 and MTH1, the DNA glycosylase MUTYH also plays a role in the repair of 8-OH-G. Mutations in *Mutyh* have been reported in patients with colorectal cancer and polyposis, a recessively inherited condition presenting with multiple colorectal adenomas [41,111,203,230]. Mice with a deletion of *Mutyh* have been shown to have a greater incidence of intestinal tumours and a greater sensitivity to potassium bromated-induced tumorigenesis [195]. MUTYH deficiency in an *Ogg1*$^{-/-}$ background has also been shown to increase the incidence of tumours in several tissues, including colon, lung, liver, heart, kidney and spleen, resulting in a significant shortening of life span relative to wild-type mice [234]. A subsequent study using the same strain of *Mutyh*$^{-/-}$, as well as *Mutyh*$^{-/-}$; *Ogg1*$^{-/-}$ mice reported that in liver, MUTYH deficiency caused an accumulation of 8-OH-G to levels similar to those observed in unstressed *Ogg1*$^{-/-}$ animals [190]. This accumulation was further increased in *Mutyh*$^{-/-}$; *Ogg1*$^{-/-}$ mice [190], suggesting a role for MUTYH in tumorigenesis in mice.

A deficiency in the BER glycosylase NTH1 has recently been ascribed a role in carcinogenesis [75]. Reduced levels of *Nth1* expression were reported in primary gastric cancers. Furthermore, two novel promoter polymorphisms were also reported to reduce *Nth1* promoter activity, but these polymorphisms were not found to be associated with an increased risk of gastric cancer [75]. A mouse model of the DNA glycosylase NTH1 has also been developed. Despite its role in BER, *Nth1*$^{-/-}$ mice did not show any increase in tumour formation or other overt phenotypic aberrations at 2 years of age [163]. It was suggested that a novel repair enzyme was compensating for lack of NTH1 in these mice [163]. An additional *Nth1*$^{-/-}$ model that was simultaneously developed also showed no phenotypic abnormalities [214]. *Nth1*$^{-/-}$ MEFs from the latter mouse model did not show increased sensitivity to oxidizing agents such as menadione or hydrogen peroxide. Furthermore, thymine glycol lesions formed in liver by x-ray irradiation were shown to be repaired, albeit more slowly in *Nth1*$^{-/-}$ mice [214]. Both mitochondrial and nuclear activities against Tg were demonstrated in liver extracts from these mice, and the existence of novel glycosylases termed TGG1 (mitochondrial and nuclear) and TGG2 (nuclear only) was proposed [214]. Similarly, whole cell extracts from another *Nth1*$^{-/-}$ mouse with no phenotypic abnormalities revealed intact BER activity against DNA containing a 5,6-dihydrouracil adduct in extracts from NTH1 deficient testis [59]. Using this latter *Nth1*$^{-/-}$ mouse model and a previously developed *Ogg1*$^{-/-}$ mouse [118], *Nth1*$^{-/-}$; *Ogg1*$^{-/-}$ mice have also been developed. Unlike the previous report from Takao et al. [214], this study did not report any residual nicking activity against Tg lesions either paired with A or G in liver mitochondrial extracts from *Nth1*$^{-/-}$ mice, possibly due to differences in purification of mitochondrial extracts as well as methods of preparing the lesion-containing oligodeoxynucleotides [115]. Regardless, no characterization of carcinogenesis was reported in these *Nth1*$^{-/-}$ *Ogg1*$^{-/-}$ mice.

The DNA glycosylase NEIL1 has also been studied for its role in repairing oxidative stress-induced DNA lesions. Inactivating mutations of NEIL1 have been reported in human gastric cancers [201]. Additionally, three rare polymorphisms have been identified in the promoter

region of *Neil1* in gastric cancers, but the functional consequences of these mutations, if any, have not yet been determined [76]. Four additional polymorphisms, including at least two inactivating mutations, have been reported for the human *Neil1* gene [188]. Knockdown of *Neil1* by antisense oligonucleotides (ASO) significantly increased the accumulation of mutations in the *Hprt* locus in both human bronchial cells and Chinese hamster ovary cells. This was further exacerbated under conditions of oxidative stress induced by treating cells with glucose oxidase for 1 hour [144]. Similarly, RNAi-mediated knockdown of *Neil1* in embryonic stem cells has been shown to render cells more sensitive to gamma irradiation [187]. *Neil1*$^{-/-}$ mice [224] and *Nth1*$^{-/-}$; *Neil1*$^{-/-}$ mice [32] have also been generated in an effort to understand the roles of these enzymes in carcinogenesis. Although very few tumours were observed in the first year of life, during the second year, both *Neil1*$^{-/-}$ and *Neil1*$^{-/-}$; *Nth1*$^{-/-}$ males developed pulmonary adenomas and carcinomas, as well as hepatocellular carcinomas at a greater rate than wild-type or *Nth1*$^{-/-}$ mice [32]. This predisposition to cancer was accompanied by a significant accumulation of Fapy-dAs and Fapy-dGs in liver, kidney and brain of NEIL1-deficient mice, without any significant increase in 8-OH-G levels [32], indicating a role for oxidative lesions other than the well-characterized 8-OH-G in tumour initiation. The *Neil1*$^{-/-}$ mouse has also been shown to have increased accumulation of mutations in mitochondrial DNA [224], and further studies on the potential mutagenicity of NEIL1 deficiency on mitochondrial DNA are warranted.

Metabolic syndrome

While most of the studies of DNA glycosylase-deficient mouse models have focused on carcinogenesis as an end point, an unexpected phenotype that has been observed in some of these mouse models is the development of features of the metabolic syndrome, including obesity and fatty liver [7,70,197,224]. In a study of *Ogg1*$^{-/-}$ mice exposed to potassium bromate, body weight analyses indicated that *Ogg1*$^{-/-}$ mice are significantly heavier than their wild-type counterparts [7]. Additionally, studies comparing various models of fatty liver disease have revealed that the severity of the fatty liver corresponds with 8-OH-G accumulation and hepatocyte death in liver and is negatively correlated with MUTYH expression in liver [70]. The most extensive evidence for a role of BER glycosylases in metabolic syndrome has come from studies of the *Neil1*$^{-/-}$ mouse model. Male *Neil1*$^{-/-}$ mice weigh significantly more than their wild-type counterparts, weighing about 50 g at 6 months of age. By 24 months of age, more than 50% of male mice weighed over 40 g, compared with only about 20% of wild-type mice [197]. This increased obesity was also accompanied by severe fatty liver, increased circulating lipids and hyperinsulinemia [224]. When subjected to an oxidative insult in the form of a high-fat diet, male *Neil1*$^{-/-}$ mice were more prone to weight gain and glucose intolerance than age-matched wild-type mice [197]. Furthermore, mtDNA content was found to be slightly reduced [197], and mtDNA damage was increased in livers of these mice [224]. Female *Neil1*$^{-/-}$ mice are also prone to obesity, albeit to a lower extent than male mice [224]. In addition to hepatic steatosis, microarray analyses of NEIL1-deficient livers revealed a robust upregulation of pro-inflammatory pathways [197]. Given the emerging link between hepatic lipid accumulation, inflammation and particular types of cancers such as hepatocellular carcinomas [134,171], the role of

NEIL1 in mediating both metabolic pathways, as well as tumorigenesis by altering inflammatory pathways warrants further investigation.

Interestingly, while both NEIL1 and OGG1 deficiency have been reported to lead to increased body weight, a recent study reported that overexpression of human OGG1 type 2a, which translocates to the mitochondria, also paradoxically leads to increased body weight in mice [237]. In this study, mice expressing the transgene were heavier and had increased hepatic lipid accumulation compared to control mice. This was largely a result of increased food intake in the transgenic mice, as pair feeding reversed the obesity phenotype in these animals. Increased food intake in these mice was accompanied by attenuated expression of the anorexigenic pro-opiomelanocortin (*Pomc*) gene and increased levels of the orexigenic agouti-related peptide (*Agrp*) gene in the hypothalamus, although the mechanisms leading to these changes are as yet unclear [237]. Mitochondrial function was not directly assessed in this study, but an increase in NADH dehydrogenase subunit 1 (ND1) mRNA, encoded by mtDNA, was reported. While the authors suggested that overexpression of OGG1 in mitochondria may lead to increased mitochondrial damage [237], this is in contrast to several reports of attenuation of oxidative damage to DNA in cell systems overexpressing OGG1 [49,84,169,178,179,189]. These prior studies were conducted by overexpressing the nuclear form of hOGG1 (Type 1a) in mitochondria. Another cell study targeting the mitochondrial 2a isoform of hOGG1 to mitochondria of *Ogg1*$^{-/-}$ MEFs reported an increase in single-strand breaks in mtDNA and consequent cell death in response to menadione treatment in these cells [166]. Therefore, it is possible that overexpression of the two different isoforms of hOgg1 may have varying consequences, and this caveat will be important in future studies evaluating the role of OGG1 activity in modulating cell function.

While the majority of the current evidence concerning the role of DNA glycosylases in the development of metabolic disease is from animal models, some reports have also confirmed this link in human populations. For instance, the Ser326Cys polymorphism of OGG1 has been linked to an increased risk for type II diabetes in a Japanese [40], as well as Mexican American population [216]. To our knowledge, a link between DNA glycosylases and body mass index in humans has not yet been reported.

Neurodegeneration

Apart from a role in tumorigenesis and metabolic disease, a limited number of studies have suggested a role for these DNA repair proteins, specifically NEIL1 and OGG1, in maintaining neuronal health. For instance, both OGG1 and NEIL1 are thought to interact with the NER protein, Cockayne Syndrome complementation group B (CSB), which is implicated in the development of Cockayne Syndrome (CS) [154,221]. Levels of 8-OH-G and FapyA/FapyG were shown to be significantly increased in brains of aged (20–24 months) *Csb*$^{-/-}$ mice [154]. Since these lesions are repaired mainly by OGG1 and NEIL1, respectively, these results were suggestive of a potential role for reduced BER in CS-induced neural damage. However, another study that crossed *Csb*$^{-/-}$ mice with *Ogg1*$^{-/-}$ mice did not observe any worsening of neurodegeneration in the double knockout animals [128], calling into question a physiological role for OGG1 in CS-induced neurodegeneration. In a different model of neuronal injury, cortical neurons from *Ogg1*$^{-/-}$ mice were shown to be more

susceptible to cell death after 6 hours of H_2O_2 treatment as compared to wild-type cells [136]. When subjected to middle cerebral artery occlusion to induce focal cerebral ischemic injury to the brain, *Ogg1*$^{-/-}$ mice had significantly larger cortical lesions than wild-type mice [136]. This was accompanied by poorer performance on a rotarod test by *Ogg1*$^{-/-}$ mice, 48 hours after the stroke, as compared to wild-type animals, suggesting that OGG1 may be involved in neuronal repair following an acute oxidative insult. Interestingly, in this study, oxidative injury led to an accumulation of OGG1 in nuclei, but not in mitochondria [136]. These data are consistent with the previously reported intracellular trafficking of OGG1 in exercise models using rat skeletal muscle [180,181]. Furthermore, reduced OGG1 levels have been shown to be associated with Alzheimer's disease in human subjects [105,146], but the mechanistic link between accumulation of 8-OH-dG and progression of Alzheimer's disease is not yet clear. In addition to Alzheimer's disease, analyses of the substantia nigra from patients with Parkinson's disease have revealed an upregulation of the mitochondrial form of OGG1 [68] and the 47-kDa form of MUTYH [4], suggesting an increased oxidative stress response in these patients. In addition to its intrinsic repair function, it has been suggested that OGG1 deficiency may also trigger release of TNFα, a pro-inflammatory cytokine that could further worsen neurodegeneration [233]. There is also some evidence that OGG1 may play a role in the progression of Huntington's disease, as reviewed by Delaney et al. in this issue [46].

Conclusion

As is evident from a review of the literature, the roles of the various BER glycosylases in modulating disease risk are complex. Much of this complexity arises from the existence of multiple enzymes that are capable of repairing the same lesions, as well as multiple isoforms of some enzymes with varying cellular localization. While the use of mouse models of either glycosylase deficiency or overexpression is a very powerful tool to understand the physiological roles of these enzymes, the animal models are not without limitations. First, the maintenance of a model deficient in DNA repair can lead to a generalized mutator phenotype, resulting in the generation of inadvertent mutations in loci other than the DNA glycosylase. Some of this can be avoided by diligent periodic backcrossing of the mutant models into a non-mutant background. In cell studies using cells derived from mutant mice, the choice of utilizing spontaneously immortalized cells versus primary cells may similarly yield conflicting results, possibly due to differences arising in the process of spontaneous immortalization in cells that are mutation prone. Additionally, many of the studies thus far have utilized mice on a mixed genetic background, usually 129SvEv and C57Bl/6. Since factors involved in the oxidative stress response, such as upregulation of antioxidant genes, are thought to be differentially modulated between these two backgrounds [209], it will be important to utilize animals that have been fully backcrossed into a uniform genetic background, in order to be able to compare various mouse models of glycosylase deficiency. Lastly, given the nuclear and mitochondrial localization of some of the glycosylases, animal models of mitochondrial- or nuclear-specific depletion of each of these enzymes will be required in order to be able to assign a causal role for any of these enzymes in disease progression. However, despite these caveats, the mouse models that have been developed

thus far provide an invaluable foundation from which to further assess the role of these DNA glycosylases in both carcinogenesis, as well as metabolic and neurologic diseases.

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