Biochemical characterization of mammalian NEIL3 and involvement in repair of hydantoin lesions in proliferative tissue

Silje Zandstra Krokeide

Thesis for the degree of Philosophiae Doctor (PhD)

Centre for Molecular Biology and Neuroscience (CMBN)
Department of Microbiology,
Oslo University Hospital
University of Oslo, Norway
2012

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 1503

ISBN 978-82-8264-435-8

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

Produced in co-operation with Akademika publishing.
The thesis is produced by Akademika publishing merely in connection with the thesis defence. Kindly direct all inquiries regarding the thesis to the copyright holder or the unit which grants the doctorate.

ACKNOWLEDGEMENT

The work presented in this thesis was carried out at the Department of Microbiology, Oslo University Hospital, Centre for Molecular Biology and Neuroscience (CMBN) and University of Oslo. The main financial supports have been provided by the Research Council of Norway and Helse Sør-Øst.

I want to express my gratitude to my supervisors. Prof. Magnar Bjørås is a great inspiration with his impressive knowledge and never ending enthusiasm to the field of DNA repair. He keeps the spirit up by always believing in my research and I never stop being impressed by how he remember the details of my gel images better than me. Dr. Luisa Luna has guided me through a whole lot of techniques and always taken her time to answer silly questions.

I am also very grateful for the inspiring and friendly working environment at the laboratory of molecular microbiology. Thank you to those who spend hours sharing knowledge about a technique without getting anything in return. Thank you to all who did not send out angry emails because someone messed up your samples. Thanks to you who shared the last tube of a reagent even if you needed every single drop. Thanks to former and present colleagues for weekend-updates and scientific discussions. You are unique and you make science extra fun!

Thank you to all co-authors of the papers included in my thesis. Dr.Yngve Sejersted, Dr. Mari Kaarbø and Dr. Toril Ranneberg-Nilsen have been super-helpful by critically reviewing this manuscript.

Finally, I will thank my dear husband Paal for showing interest in my field (Where did your gel run today?) and for making my life complete. Ask and Storm, our two little ones, keeps us busy and happy.

CONTENTS

LIST OF PAPERS

I **Expression and purification of NEIL3, a human DNA glycosylase homolog**
Silje Z. Krokeide, Nils Bolstad, Jon K. Laerdahl, Magnar Bjørås and Luisa Luna
Protein Expression and Purification 2009 Jun;65(2):160-4

II **Human NEIL3 is mainly a monofunctional DNA glycosylase with affinity to spiroiminodihydantoin and guanidinohydantoin**
Silje Z. Krokeide, Medya Salah, Jon K. Laerdahl, Luisa Luna, F. Henning Cederkvist, Aaron M. Fleming, Cynthia J. Burrows, Bjørn Dalhus, and Magnar Bjørås
Manuscript submitted to DNA repair

III **Loss of Neil3, the major DNA glycosylase activity for removal of hydantoins in single-stranded DNA, reduces cellular proliferation and sensitizes cells to genotoxic stress**
Veslemøy Rolseth, Silje Zandstra Krokeide, David Kunke, Christine Gran Neurauter, Rajikala Suganthan, Yngve Sejersted, Gunn Annette Hildrestrand, Magnar Bjørås and Luisa Luna
Manuscript submitted to BBA Molecular Cell Research

IV **Endonuclease VIII-like 3 (Neil3) DNA glycosylase promotes neurogenesis induced by hypoxia-ischemia**
Yngve Sejersted, Gunn Annette Hildrestrand, David Kunke, Veslemøy Rolseth, Silje Zandstra Krokeide, Christine Gran Neurauter, Rajikala Suganthan, Monica Atneosen-Åsegg, Aaron M. Fleming, Ola Didrik Saugstad, Cynthia J. Burrows, Luisa Luna, and Magnar Bjørås
Proceedings of the National Academy of Sciences of the United States of America (PNAS). 2011 Nov15;108(46): 18802-7

ABBREVIATIONS

5-OHC	5-hydroxycytosine
5-OHU	5-hydroxyuracil
8-oxoG	7,8-dihydro-8-oxoguanine
A	adenine
AAG	alkyladenine DNA glycosylase
AD	Alzheimer's disease
AGT	O6-methylguanine-DNA methyltransferase
AlkB	alpha-ketoglutarate-dependent dioxygenase B
ALS	amyotrophic lateral sclerosis
AP	apurinic/apyrimidinic
APE	AP endonuclease
AT	ataxia telangiectasia
ATP	adenosine triphosphate
BER	base excision repair
BrdU	bromodeoxyuridine
C	cytosine
CFU	colony forming unit
CNS	central nervous system
CP	Cockayne syndrome
DG	dentate gyrus
DNA	deoxyribonucleic acid
DR	direct reversal
dRP	deoxyribose phosphate
DSB	double-strand break
dsDNA	double-stranded DNA
E.coli	*Escherichia coli*
FaPy	formamidopyrimidine
FEN1	flap endonuclease 1
FL	full-length
Fpg	formamidopyrimidine DNA glycosylase
G	guanine
GD	glycosylase domain
GFAP	glial fibrillary acidic protein
Gh	guanidinohydantoin
GPS	glial progenitor cell
H2TH	helix-two turn-helix
HD	Huntington's disease
HhH	helix-hairpin-helix
HEK	human embryonic kidney cell
HI	hypoxia-ischemia
HIF1α	hypoxia-inducible factor 1α
HIV	human immunodeficiency virus
HNPCC	hereditary non-polyposis colorectal cancer
HR	homologous recombination
kDa	kilodalton
KO	knockout
LIG I	DNA ligase I
LIG III	DNA ligase III
LP-BER	long-patch BER
MAP	MUTHY-associated polyposis
MBD4	methyl-CpG binding domain 4 glycosylase

MEF	mouse embryonic fibroblast
MGMT	O6-methylguanine-DNA methyltransferase
MMR	mismatch repair
MPG	3-methyladenine DNA glycosylase
mRNA	messenger RNA
MS	mass spectrometry
MUTYH	mutY homologue
NBS	Nijmegen breakage syndrome
Nei	endonuclease VIII
NEIL	endonuclease VIII-like
NER	nucleotide excision repair
NHEJ	non-homologous end joining
NLS	nuclear localization signal
NPC	neural progenitor cell
NSC	neural stem cell
NSPC	neural stem/progenitor cell
NTH1	endonuclease III
OGG1	8-oxoguanine DNA glycosylase
P	phosphate
P38 MAPK	p38 mitogen-activated protein kinase
PCNA	proliferating cell nuclear antigen
PD	Parkinson's disease
PNK	polynucleotide kinase
POL	polymerase
PTM	post translational modification
PUA	phospho a,ß unsaturated aldehyde
RanBP	ran binding protein
RNA	ribonucleic acid
ROS	reactive oxygen species
RPA	replication protein A
RING1	ring finger protein 1
S	synthesis phase during cell cycle
SAM	s-adenosylmethionine
SGZ	subgranular zone
siRNA	small interfering RNA
SMUG1	single-strand selective monofunctional uracil DNA glycosylase
SNP	single nucleotide polymorphism
Sp	spiroiminodihydantoin
SP-BER	short-patch BER
SSB	single-strand break
SSB	single-strand binding protein
ssDNA	single-stranded DNA
SVZ	subventricular zone
T	thymine
Tg	thymine glycol
TDG	thymine DNA glycosylase
TTD	trichothiodystrophy
U	uracil
UDG	uracil DNA glycosylase
UNG	uracil DNA N-glycosylase
UV	ultraviolet
WRN	Werner helicase
XP	xeroderma pigmentosum
XRCC1	X-ray repair cross-complementing protein 1

SUMMARY

Oxidative damage is a major threat to the integrity of our genome and can lead to mutations or block replication if not repaired. Base excision repair (BER) is the main pathway for repair of oxidative lesions and is initiated by the action of a DNA glycosylase. A monofunctional glycosylase recognizes and excises the modified nucleotide, while a bifunctional glycosylase in addition possesses an intrinsic apurinic/apyrimidinic (AP) lyase activity generating a nick in the DNA strand. There are five DNA glycosylases acting on oxidative damage in eukaryotic cells; 8-oxoguanine DNA glycosylase (OGG1), endonuclease III (NTH1), and endonuclease VIII-like 1, 2 and 3 (NEIL1, NEIL2 and NEIL3). These enzymes are bifunctional with overlapping substrate specificity and knockout mouse studies reveals that none of them are essential for life. NEIL3 is sequentially related to the well characterized enzymes NEIL1 and 2, but less is known about its biochemical properties. Paper I of this thesis includes an expression and purification protocol for human NEIL3. NEIL3 recognizes and excises guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) from the genome. In paper II we elucidate the amino acids important for activity. NEIL1 and NEIL2 have tightly coupled DNA glycosylase/AP lyase activities and perform β,δ-elimination mechanism to incise DNA. NEIL3 has uncoupled activities, where the base excision is more efficient than the strand incision. We demonstrate that a V2P mutation changes the catalytic properties of NEIL3 to be similar to the activity of NEIL1 and 2. While OGG1, NTH1, NEIL1 and NEIL2 are expressed in most tissues throughout the organism, NEIL3 expression is limited to some lymphatic organs and compartments of the brain harboring neural stem cells, indicating a role in highly proliferative tissue. Paper III shows that mouse Neil3 is responsible for repair of Gh and Sp lesions in single-stranded DNA (ssDNA) in thymus and spleen, where repair activity correlates with Neil3 expression level. In paper IV of this study, we expose young Neil3-deficient mice to hypoxia-ischemia, to introduce cell death and activate proliferation of neural tissue. We find that the Neil3 deficit leads to reduced regeneration of neural tissue in the striatum. Further, *in vitro* propagated Neil3-deficient neural stem cells have a depressed neurogenesis and impaired ability to repair Gh and Sp lesions in ssDNA. Taken together, we suggest that NEIL3 has a unique role in removing hydantoin lesions from ssDNA and thereby promoting replication of proliferating cells.

INTRODUCTION

'We totally missed the possible role of … [DNA] repair although… I later came to realize that DNA is so precious that probably many distinct repair mechanisms would exist.' Francis Crick, in Nature 1974 (Crick, 1974).

When the molecular structure of DNA was presented by James Watson and Francis Crick in 1953 (Watson J & Crick, 1953) it was believed to be extraordinary stable in order to maintain the genetic information, and to pass it on for generations. Decades later it was clear that our DNA is very vulnerably for damage. It has been estimated that a single cell can suffer from up to a million lesions in one day (Lodish *et al*, 2004). Damage can arise after environmental conditions like exposure to strong sunlight and uptake of toxins through air or food, they can arise as a consequence of natural cellular processes like the metabolism, or they can arise spontaneously by nucleotide deamination and replication errors. In contrast to other biomolecules, DNA cannot be replaced, only repaired.

From an evolutionary point of view, the instability of DNA is the source of diversity and natural selection. From a medical point of view, it is a constant threat to the organism. A

large number of cancers and genetic diseases are linked to insufficient repair of damaged DNA. DNA damage accumulates with age and common for several of the repair-associated diseases are the phenotypes of premature aging (Niedernhofer *et al*, 2006). All eukaryotic cells are equipped with a multifaceted damage response system to counteract with the deleterious effects of DNA damage (figure 1). Sensing of lesions activates cell cycle checkpoints and arrests cell cycle progression to give time for DNA repair. Replication of damaged DNA can lead to fixation of mutations. To encounter the plethora of different DNA lesions, an intricate network of repair through different pathways has evolved. If the extent of damage is too severe to be repaired, the cell may commit suicide by entering apoptosis, for the benefit of the organism. Cells are also equipped with damage-tolerant translesion DNA polymerases bypassing a broad range of lesions, allowing DNA replication to continue. However, translesion polymerases have a high propensity to insert wrong bases, generating mutations which are fixated upon further replication cycles. Such accumulation of mutations is one of the main mechanisms leading to cancer (Zahn *et al*, 2011; Venitt, 1996). DNA repair mechanisms are found in all organisms and many of the enzymes are highly conserved from bacteria to man.

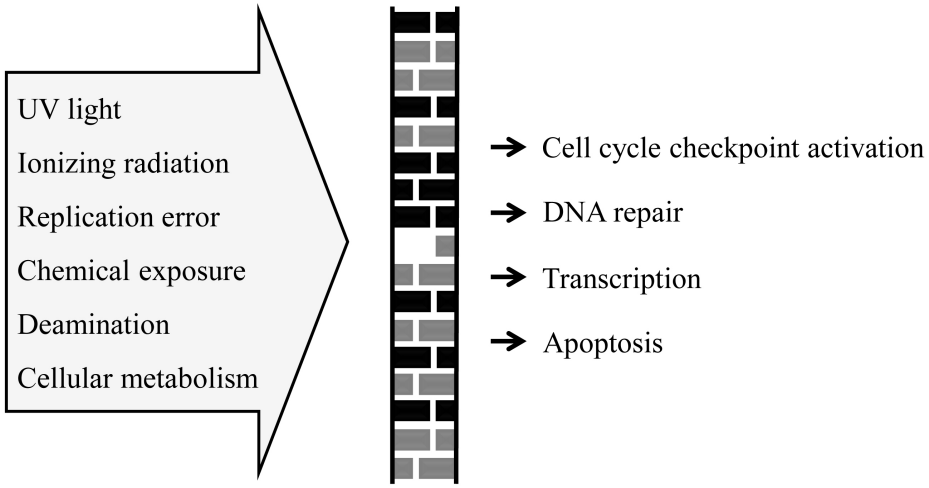


Figure 1. DNA damage activates the DNA damage response. Different exogenous and endogenous agents can cause DNA damage which triggers the DNA damage response, leading to cell cycle checkpoint activation. Successful repair of the lesions leads to prolonged cell cycle progression and transcription, while the cell will enter apoptosis if the extent of the damage is too severe to be repaired.

Endogenous and exogenous sources for DNA damage

Living organisms are constantly exposed to various environmental agents, producing a variety of DNA lesions. An example is ultraviolet (UV) light inducing bulky dipyrimidine photoproducts in DNA. Extensive UV-exposure from the sun or from sun beds can lead to skin cancer (Kanavy & Gerstenblith, 2011). Another exogenous threat to genome integrity is ionizing radiation from cosmic rays, naturally occurring radioactive material, X-rays as well as artificially produced radioisotopes. Ionizing radiation can liberate electrons from molecules, causing a broad range of DNA lesions including damage on bases and sugars, interstrand crosslinks, DNA single-strand breaks (SSBs), and DNA double-strand breaks (DSBs) (Morgan & Sowa, 2005; Ward, 1988). DNA damaging compounds can also be introduced through air, water or food. Alkylating agents have potential to transfer alkyl groups onto DNA. A pronounced example is cigarette smoking, which kills 1,5 million people every year due to lung cancer caused by premutagenic DNA lesions (Proctor, 2012). Humans can to some extent protect themselves against the environmental mutagens, but damage from endogenous sources is unavoidable. An example of an endogenous alkyl donor is S-adenosylmethionine (SAM), a metabolite involved in many biochemical reactions. The high transfer potential of this methyl donor makes it prone to spontaneously methylate DNA (Xiao & Samson, 1993; Rydberg & Lindahl, 1982; Taverna & Sedgwick, 1996). Both N and O atoms in the DNA structure can be alkylated, leading to premutagenic and cytotoxic lesions. Water is another endogenous threat to our genome. The base-sugar bonds of DNA are vulnerable to hydrolytic attack, leading to apurinic/apyrimidinic (AP) sites, which are highly cytotoxic and can lead to SSBs if not repaired. DNA bases are estimated to be lost by hydrolysis at a rate of 10 000 per cell per day, whereas 95 % of these baseless sites are apurinic (Lindahl & Nyberg, 1972; Lindahl, 1993; Kasai *et al*, 1984). Abasic sites stall DNA polymerase and are also highly mutagenic at transcription level, because of the potential of RNA polymerase to bypass the lesion by introducing an adenine for the missing nucleotide (Dahlmann *et al*, 2010; Zhou & Doetsch, 1993; Clauson *et al*, 2010). The amine groups on the nucleotides are also exposed to hydrolytic attack which leads to a single base mutation. Cytosine, 5-methylcytosine, guanine and adenine are deaminated to uracil, thymine, xanthine and hypoxanthine, respectively. DNA is much more prone to deamination when it is in single-stranded stages like fork and bubble structures (Lindahl, 1993), suggesting most of the deamination to occur during transcription or replication.

ROS and oxidative DNA damage

Oxidative DNA damage results from reaction with reactive oxygen species (ROS). ROS are produced from endogenous and exogenous sources and can chemically modify the DNA backbone as well as both pyrimidine and purine bases. Oxidative DNA damage includes single- and double strand breakage, DNA-protein cross-links, oxidized AP sites, base deamination products, oxidized sugar fragments and a plethora of oxidized DNA bases (van Loon *et al*, 2010).

Cellular metabolism leads to constant generation of ROS as a by-product of oxidative phosphorylation in mitochondria. Electrons from organic molecules (from food) are transported through the chain of three large protein complexes in the mitochondrial inner membrane and delivered to oxygen, leading to formation of H_2O and the cellular energy storage adenosine triphosphate (ATP). Most of the oxygen respired is used to achieve the electrons in the end of the electron transport chain, some of the oxygen molecules will react with electrons before they have completed their journey through the electron transport chain, leading to the formation of the superoxide radical (O_2•-) (Valko *et al*, 2007). Superoxide is not as reactive itself, but can be converted to the highly reactive hydroxyl radical (•OH). Another endogenous source of ROS is peroxisomal metabolism. Peroxisomes are organelles present in all eukaryotic cells, involved in the β-oxidation of fatty acids. Peroxisome oxidases remove hydrogen atoms from fatty acids, by the introduction of oxygen to form hydrogen peroxide (H_2O_2). H_2O_2 is further used by the enzyme peroxidase to oxidize other substrates, including alcohol, phenols and formic acids present in the cell (Schrader & Fahimi, 2006;Wanders & Waterham, 2006). This reaction is especially important in the kidneys and the liver where peroxisomes detoxify various toxic substances that enter the blood. The equilibrium between production and scavenging of ROS in the peroxisomes is a fine balance, and disconcert contributes to the total cellular generation of ROS. H_2O_2 is not as reactive itself, but can lead to production of •OH in a process called the Fenton reaction (Wardman & Chandeias, 1996). ROS is also formed during the oxidative burst triggered during inflammatory responses. During an infection, cells from the immune system will overproduce both nitric oxide (NO•) and superoxide (O_2•-) which may react to produce peroxynitrite anion ($ONOO^-$), an extremely potent oxidizing agent (Ahmad *et al*, 2009; Burney *et al*, 1999).

Moreover, ROS can arise from exogenous sources. Ionizing radiation and UV-light can produce oxygen radicals by hydrolysis of water (Riley, 1994; Black, 1987).

Cells are equipped with enzymes to disarm free radicals. Catalase and glutathione peroxidase catalyze the breakdown of H_2O_2 and superoxide dismutase catalyzes the destruction of $O_2^{\bullet-}$ (Salganik, 2001). Although most of the free radicals are neutralized by cellular enzymes, residual ROS is the major endogenous toxin in the aerobic cell.

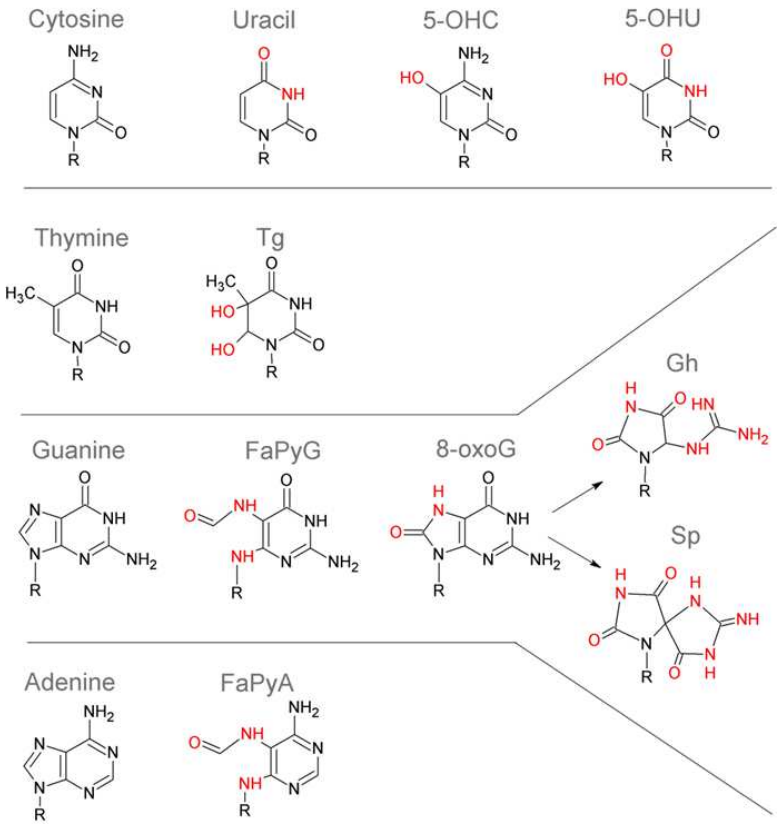


Figure 2. Examples of oxidative base lesions. Cytosine can be oxidized to 5-hydroxycytosine (5-OHC) or be deaminated into uracil, which can form the oxidation product 5-hydroxyuracil (5-OHU). Thymine can be oxidized into thymine glycol (Tg). Guanine can form the formamidopyrimidine (FaPy) lesion FaPyG, or it can be oxidized to 8-oxoguanine (8-oxoG), which can further form guanidinohydantoin (Gh) or spiroiminodihydantoin (Sp). Adenine can be modified to FaPyA. Changes from undamaged nucleotide are marked in red.

Up to date, there are identified more than 100 different types of oxidative base lesions in the mammalian genome. Some of the most studied lesions are illustrated in figure 2. The pyrimidines cytosine, uracil and thymine can be modified to 5- hydroxycytosine (5-OHC), 5-hydroxyuracil (5-OHU) and thymine glycol (Tg), respectively. The purines guanine and adenine can form the formamidopyrimidine (FaPy) lesions FaPyG and FaPyA. Guanine has a low redox potential, which makes it especially vulnerable to oxidation. 7,8-dihydro-8-oxo-guanine (also known as 8-oxoguanine, 8-oxoG) is formed in large quantities and is the most thoroughly examined oxidation product. 8-oxoG is often used as a marker to indicate oxidative stress in cells (Klaunig *et al*, 2011). This lesion is mutagenic due to its ability to pair with adenine as well as cytosine leading to a G → T mutation upon replication (Shibutani *et al*, 1991; Cheng *et al*, 1992). 8-oxoG has a lower redox potential than guanine and is prone for further oxidation, leading to a variety of lesions including the hydantoin products guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) (Duarte *et al*, 1999; Munk *et al*, 2008).

Repair mechanisms in mammalian cells

To counteract all the different types of damage to DNA, the cell has evolved multiple DNA repair mechanisms, which can be divided into six major pathways (figure 3): Nucleotide excision repair (NER), mismatch repair (MMR), base excision repair (BER), nonhomologous end joining (NHEJ), homologous recombination (HR) and direct reversal (DR).

NER removes a large variety of helix-distorting lesions caused by mutagenic chemicals, chemotherapeutic drugs or UV-radiation (Vermeulen, 2011; Hoeijmakers, 2009). More than 30 proteins are involved in the mammalian NER pathway. NER is divided into two subpathways. The global genome NER detects and repairs lesions in the entire genome while the transcription coupled NER removes lesions from the transcribed strands of active genes. Three genetic disorders are associated with defective NER; xeroderma pigmentosium (XP), trichothiodystrophy (TTD), and Cockayne syndrome (CP). All three diseases are associated with hypersensitivity to the sun as a consequence of the impaired ability to repair lesions caused by UV-radiation. The relative risk for a person with XP to develop skin cancer is 2000 times higher than for an healthy individual (Diderich *et al*, 2011; Hoeijmakers, 2009; Niedernhofer *et al*, 2011).

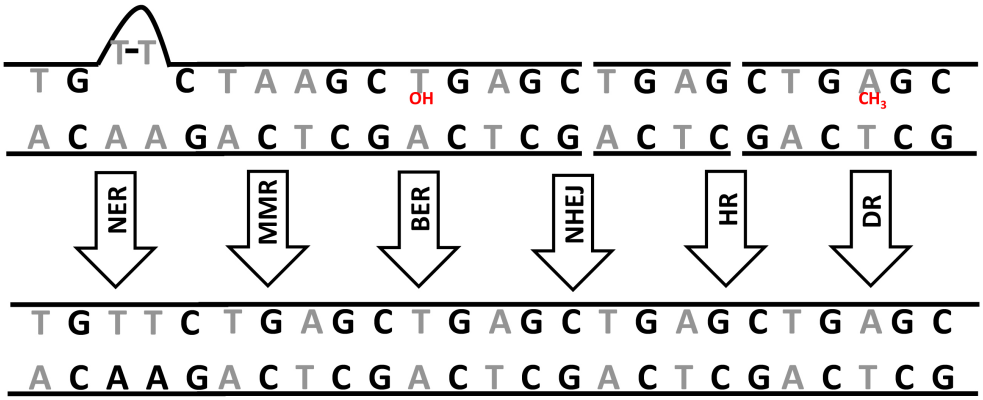


Figure 3. Overview of repair mechanisms in mammalian cells. Nucleotide excision repair (NER) repairs bulky DNA lesions like pyrimidine dimers caused by UV-exposure. Mismatch repair (MMR) excises and replaces mispaired nucleotides. Base excision repair (BER) excises and replaces bases with minor modifications. Nonhomologous end joining (NHEJ) and homologous recombination (HR) repairs double strand breaks. Direct reversal (DR) repairs alkylated bases.

The MMR pathway exchanges bases that are mispaired by DNA polymerases and removes insertion/deletion loops introduced during replication (Jiricny, 2006). MMR can distinguish between the template and the newly synthesized strand and will excise the misincorporated nucleotide in a process where 100-1000 nucleotides are removed. Defects in the MMR pathway drastically enhances the mutation frequency and can lead to sporadic tumors. Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) is a genetic condition with impaired MMR. Nine out of ten HNPCC patients develope cancer during lifespan (Vasen *et al*, 1996).

The BER pathway is a multistep process repairing small, nonbulky alterations in DNA including single base lesions, AP sites and SSBs (Dalhus *et al*, 2009; Robertson *et al*, 2009). BER is the main pathway for repair of damage caused by ROS and will be described in more detail in the next section.

HR and NHEJ are the pathways for repair of DSBs (Kasparek & Humphrey, 2011; Hartlerode & Scully, 2009). HR is the preferred pathway for DSB repair in bacteria and yeast and requires an identical DNA copy to complete repair. NHEJ repairs 90 % of the DSBs in

mammalian cells and directly ligates the ends of the strand breaks without the need for a homologous template. NHEJ is also functioning during development of T- and B-cell receptors through V(D)J recombination. Defects in HR and NHEJ repair can result in chromosomal instability leading to somatic mutations. Ataxia telangiectasia (AT) and Nijmegen breakage syndrome (NBS) are two genetic human diseases caused by defects in the DSB repair. Both diseases are characterized by neuronal degeneration with loss of brain function, immunodeficiency and high risk of cancer (Thompson & Schild, 2002).

DR is a repair mechanism which in contrast to the other repair pathways, only involves one enzyme and does not involve cleavage of the DNA backbone or the damaged nucleotide (Eker *et al*, 2009; Drabløs *et al*, 2004). There are three enzymes performing DR identified in mammals. O^6-methylguanine-DNA methyltransferase (MGMT/AGT) repairs O^6-methylguanine lesions by the transfer of a methyl group from the lesion to a cysteine group in the active site, thereby inactivating itself. MGMT has been shown to be involved in repair of alkylating damage caused by several anti-cancer chemotherapeutic drugs (Verbeek *et al*, 2008), and MGMT-deficient mice are hypersensitive to this type of treatment (Iwakuma *et al*, 1997). Alpha-ketoglutarate-dependent dioxygenase B (AlkB) homologues 2 and 3 (ABH2 and ABH3) repair 1-methylguanine and 3-methylcytosine by oxidative demethylation (Falnes *et al*, 2007). Photolyases are DR enzymes repairing UV induced pyrimidine dimers in DNA by the use of energy from light. Photolyases are found in all kingdoms of life, without placental mammals, where pyrimidine dimers are repaired by the NER pathway (Brettel & Byrdin, 2010).

Base excision repair

The short-patch pathway for BER (SP-BER) requires the function of four proteins as demonstrated in figure 4. The pathway is initiated by a DNA-glycosylase that recognizes and excises the damaged base by cleavage of the N-glycosylic bond, creating an AP site. The DNA glycosylases are divided into two classes, based on their mechanism; they can be monofunctional or bifunctional. A monofunctional glycosylase recruits an AP endonuclease (APE1) to the abasic site. APE1 creates a nick 5’ to the baseless site. The remaining 5’ – deoxyribose phosphate (5’dRP) termini is trimmed by the lyase activity of DNA polymerase

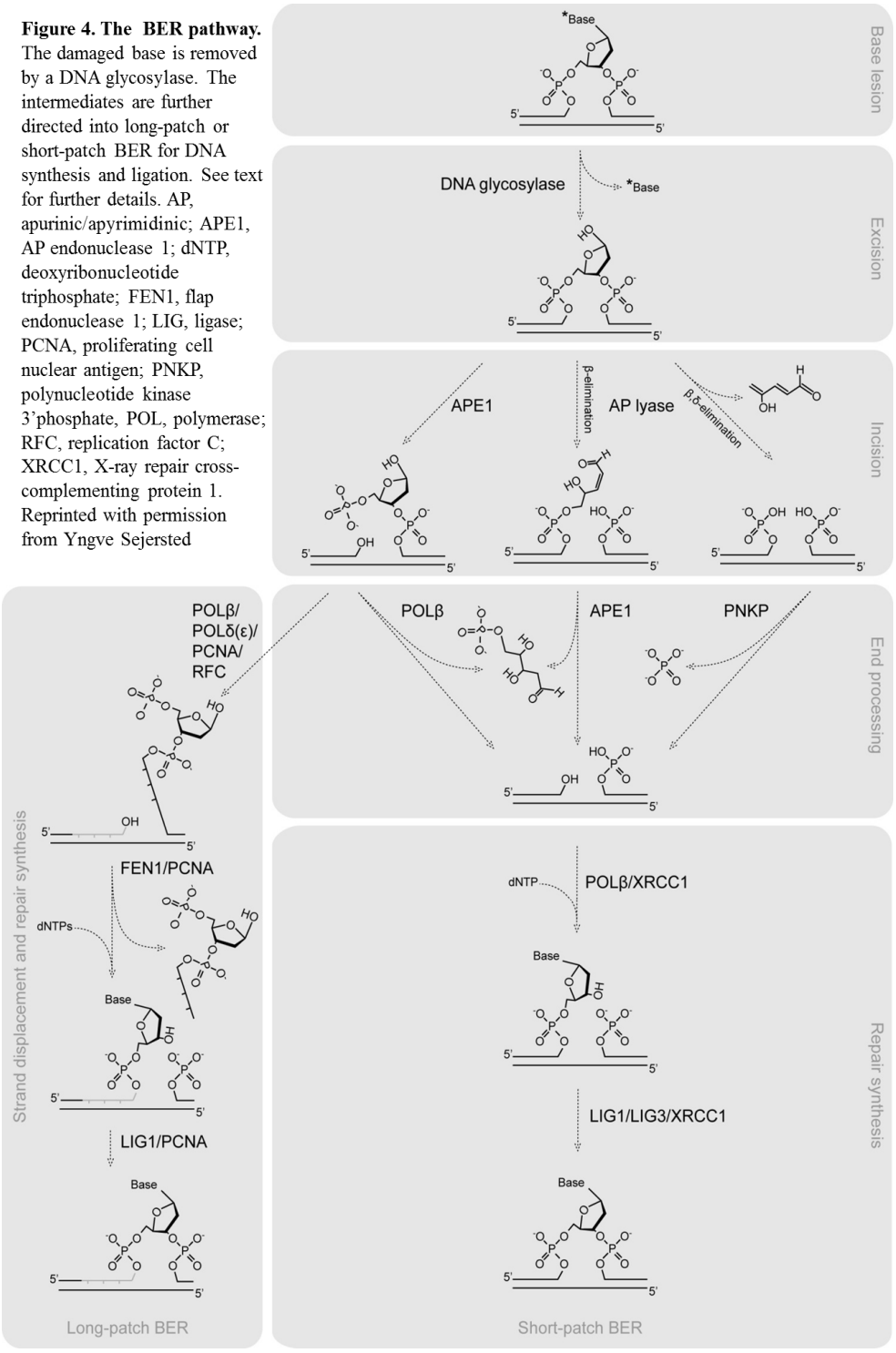


Figure 4. The BER pathway. The damaged base is removed by a DNA glycosylase. The intermediates are further directed into long-patch or short-patch BER for DNA synthesis and ligation. See text for further details. AP, apurinic/apyrimidinic; APE1, AP endonuclease 1; dNTP, deoxyribonucleotide triphosphate; FEN1, flap endonuclease 1; LIG, ligase; PCNA, proliferating cell nuclear antigen; PNKP, polynucleotide kinase 3'phosphate, POL, polymerase; RFC, replication factor C; XRCC1, X-ray repair cross-complementing protein 1. Reprinted with permission from Yngve Sejersted

β (POL β) (Matsumoto & Kim, 1995; Prasad *et al*, 1998) and the single nucleotide gap is filled with the correct base with POL β (Sobol *et al*, 1996). DNA ligase III (LIG III) bound to the non-enzymatic scaffold protein, X-ray repair cross-complementing protein 1 (XRCC1), completes the repair process by sealing the nick (Tomkinson & Mackey, 1998; Kubota *et al*, 1996; Caldecott *et al*, 1996; Campalans *et al*, 2005). However, recent work has demonstrated that LIG III is essential for mitochondrial BER but not for nuclear BER, where DNA ligase 1 (LIG I) completes repair when LIG III is absent (Gao *et al*, 2011; Simsek *et al*, 2011). A bifunctional glycosylase has an intrinsic lyase activity. In addition to removal of the damaged base, it incises the DNA strand 3' to the AP site by either β-elimination or β,δ –elimination (Bailly & Verly, 1987; Bailly *et al*, 1989). The resulting phospho α,β-unsaturated aldehyde (PUA) or the phosphate group (P) attached to the 3' end of the nick are removed by the diesterase activity of APE1 or the phosphatase activity of polynucleotide kinase (PNK) respectively, to create 3' OH ends in order to be further processed by POL β and LIG I/LIG III (Wiederhold *et al*, 2004; Chen *et al*, 1991).

While the POL β in SP-BER replaces one nucleotide, the DNA polymerases in the long patch BER (LP-BER) continues the syntheses until two to 13 nucleotides are reinstated 3' of the lesion (Wallace *et al*, 2012). Different DNA polymerases (POL β, ε, δ, λ, ι, and θ) are found to be involved in strand displacement in different subpathways of LP-BER (Brown *et al*, 2011). There is still a lot to be unraveled about the choice and coordination of the different DNA polymerases in long patch BER. The flap structure generated by the DNA polymerase is removed by flap endonuclease 1 (FEN1) and the nick is sealed by LIG I. Proliferating cell nuclear antigen (PCNA) is also involved in long patch BER by interaction with DNA polymerases, FEN1 and LIG 1, supporting their functions (Klungland & Lindahl, 1997; Matsumoto *et al*, 1999; Pascucci *et al*, 1999). The choice between short patch and long patch BER seems to be dependent on type of lesion, chemistry of the abasic site, cell cycle stage as well as sub nuclear localization of the repair complex (Fortini & Dogliotti, 2007).

DNA glycosylases

BER is initiated by a DNA glycosylase, the key enzyme of the BER pathway. At the time writing, there are 11 DNA glycosylases identified in mammals. Many of these enzymes have

a broad and overlapping substrate range demonstrated by *in vitro* experiments, and they are believed to step in for each another *in vivo* (Hoeijmakers, 2001; Dalhus *et al*, 2009; Friedman & Stivers, 2010). DNA glycosylases are all monomeric proteins binding non-specific to the negatively charged DNA through hydrogen bonds and salt bridges. They move along the helix in their search for DNA damage, often only a minor base modification in the vast excess of normal DNA. In this searching process, the DNA glycosylase probably switches between fast mode, tracking along either one of the DNA grooves or the sugar-phosphate backbone, and slow mode where the DNA glycosylase intrudes and distorts the helix, creating a wide and flat minor groove (Banerjee *et al*, 2006; Dunn *et al*, 2011; Porecha & Stivers, 2008; Blainey *et al*, 2006, 2009). If a lesion is recognized, the DNA glycosylase extrudes the damaged nucleotide from the helix by rotating it around the flanking phosphodiester bonds. This strategy, known as base-flipping, increases the surface for molecular interactions and catalyzes the base release (Friedman & Stivers, 2010; Slupphaug *et al*, 1996; Roberts & Cheng, 1998). A residue from the active site of the enzyme will release the damaged base by nucleophilic attack of the anomeric C1' carbon.

DNA glycosylases can be classified into structural classes/superfamilies based on their 3D structure (Fromme *et al*, 2004) (Table 1). The uracil DNA glycosylase (UDG) superfamily is a group of monofunctional DNA glycosylases. Uracil DNA N-glycosylase (UNG), single-strand selective monofunctional uracil DNA glycosylase (SMUG1) and thymine DNA glycosylase (TDG) are the mammalian enzymes in this class. They are small single domain proteins, all recognizing uracil bases in DNA. UNG is well characterized and is highly conserved from bacteria to man. SMUG1 can also remove 5-hydroxymethyluracil from DNA, while TDG is a mismatch DNA glycosylase and can in addition to uracil recognize thymine mismatched to a guanine (Pearl, 2000; Visnes *et al*, 2009). Methyl-CpG-binding domain protein 4 (MBD4) is the other mismatch DNA glycosylase found in mammals, it removes uracil and thymine resulting from deamination of CpG and methylated CpG respectively (Hendrich *et al*, 1999). MBD4 is a member of the helix-hairpin-helix (HhH) superfamily, which is termed after its distinct conserved motifs (Mullen & Wilson, 1997). Mut Y homologue (MUTYH), OGG1 and NTH1 are the other mammalian DNA glycosylases with the HhH motif. They are all two-domain proteins with the active site at the junction. The damaged nucleotide is displaced by nucleophilic attack from a lysine residue (Dalhus *et al*, 2009).

Table 1. Mammalian DNA glycosylases

Superfamily[a]	Protein	Reaction mechanism[b]	Substrates[c]
UDG	UNG	Monofunctional	U, 5-FU
	SMUG1	Monofunctional	U, 5OH-meU
	TDG	Monofunctional	T, U or εC opposite G (CpG sites)
HhH	MBD4	Monofunctional	T or U opposite G at CpG, T opposite O6-meG
	MUTHY	Monofunctional	A opposite 8-oxoG, 2OHA opposite G
	NTH1	Bifunctional (β), coupled activities	Tg, DHU, FaPyG, 5OHU, 5OHC, Sp, Gh (dsDNA only)
	OGG1	Bifunctional (β), uncoupled activities	8-oxoG opposite C, FaPyG (ds DNA only)
AAG	AAG/MPG	Monofunctional	3-meA, hypoxanthine, εA
H2TH	NEIL1	Bifunctional (β,δ) coupled activities	Tg, DHU, DHT, FaPyG, FaPyA, 5OHU, 5OHC, 8-oxoG, Sp, Gh
	NEIL2	Bifunctional (β,δ) coupled activities	Overlap with NEIL1 with some minor differences
	NEIL3	Bifunctional (β), uncoupled activities	FaPyG, FaPyA, Sp, Gh

[a](Fromme *et al*, 2004), [b](M. L. Hegde, Tapas K Hazra, et al. 2008, Paper II of this thesis), [c](Barnes & Lindahl, 2004; Morland *et al*, 2002; Liu *et al*, 2010; Dou *et al*, 2003a; Jacobs & Schär, 2012; Krishnamurthy *et al*, 2008; Redrejo-Rodríguez *et al*, 2011; Zhao *et al*, 2010; Robertson *et al*, 2009)

MUTYH is a monofunctional enzyme, and is the only DNA glycosylase that removes adenine incorporated opposite 8-oxoG, however this misincorporation can also be repaired by enzymes from the MMR pathway (Russo *et al*, 2007). OGG1 removes oxidized purines and is the major DNA glycosylase for removal of 8-oxoG (Bjorâs *et al*, 1997). NTH1 is highly conserved among species and repairs a broad spectrum of oxidized pyrimidines (Dizdaroglu *et al*, 1999). The AAG superfamily only contains one known mammalian DNA glycosylase, the alkyladenine DNA glycosylase (AAG/MPG), a single domain enzyme recognizing alkylated bases in DNA (Lau *et al*, 1998). The fourth and last structural class, the helix-two-turn-helix (H2TH) superfamily, contains three mammalian enzymes; NEIL1, NEIL2 and NEIL3 (Prakash *et al*, 2012).

NEIL1, NEIL2 and NEIL3

The H2TH superfamily had until ten years ago, only known members from the bacterial kingdom, termed endonuclease VIII (Nei) and formamidopyrimidine DNA glycosylase (Fpg) (Chetsanga & Lindahl, 1979; Melamede *et al*, 1994; Sugahara *et al*, 2000). Before then, only two mammalian DNA glycosylases repairing oxidative lesions, OGG1 and NTH1, were known. However, studies in Ogg1 and Nth1 deficient mice revealed the existence of other enzymes repairing oxidative DNA lesions (Klungland *et al*, 1999; Minowa *et al*, 2000), leading to the search for new DNA glycosylases. The endonuclease VIII-like (NEIL) glycosylases NEIL1, NEIL2 and NEIL3 (figure 5) were reported by us and others in 2002,(Morland *et al*, 2002; Bandaru *et al*, 2002; Hazra *et al*, 2002b, 2002a; Takao *et al*, 2002b, 2002a).

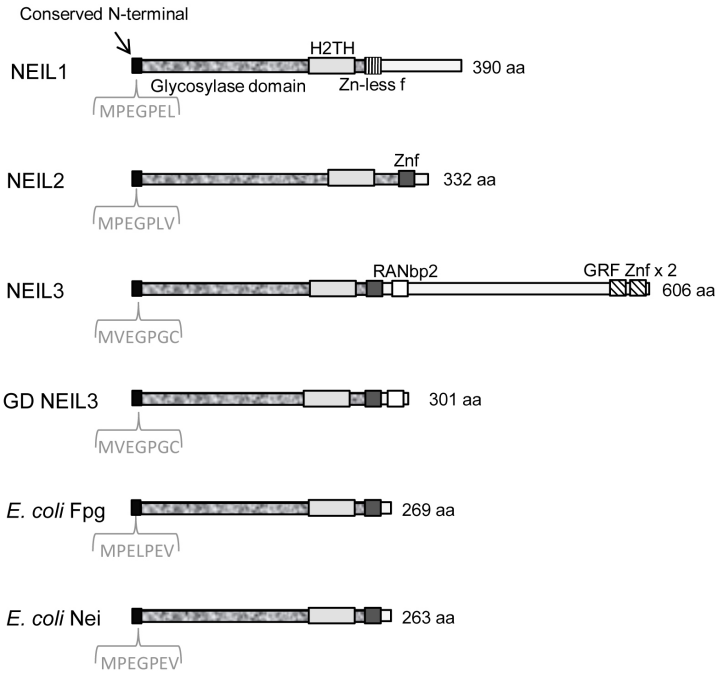


Figure 5. Schematic presentation of mammalian NEIL1, NEIL2, NEIL3 and *E. coli* Nei and Fpg. The first amino acids in the N-terminal, required for catalytic activity and the helix-two-turn-helix motif (H2TH) are conserved within the superfamily. NEIL1 has a Zn-less finger domain and NEIL2, NEIL3, Fpg and Nei have a conserved Zn finger.NEIL3 has an additional RAN binding protein (RANbp) Zn finger, a nuclear localization sequence (NLS) and a unique structurally disordered C-terminal with two GRF Zn fingers.

The highly conserved active site in the H2TH superfamily is found in the first amino acids of the N-terminal glycosylase domain (GD). The mammalian NEIL1 and NEIL2, and the prokaryote Fpg and Nei contain a Pro2, responsible for the nucleophilic attack of the N-glycosylic bond of the damage nucleotide. In NEIL3, this residue is replaced by valine (Takao *et al*, 2002b; Morland *et al*, 2002). In *E. coli*, Nei mainly excises oxidized pyrimidines (Wallace *et al*, 2003), and Fpg mainly recognizes oxidized purines and FaPy lesions (Tchous *et al*, 1994). NEIL1 and NEIL2 have been well characterized with respect to activity and their preferred substrates are oxidized pyrimidines including the hydantoin lesions Sp and Gh, Tg, FaPyA, FaPyG and 5-OHC (Hazra *et al*, 2002a; Morland *et al*, 2002; Rosenquist *et al*, 2003; Hailer *et al*, 2005a) as well as 5-OHU and 8-oxoG in bubble structures (Dou *et al*, 2003b). NEIL1 and NEIL2 recognize most of the same lesions, but with minor variations. NEIL3 has overlapping substrate range to NEIL1 and 2, with the main substrates being FaPyA, FaPyG and the hydantoin lesions Sp and Gh (Liu *et al*, 2010).

In addition to the N-terminal domain responsible for the DNA glycosylase/AP lyase activity and the H2TH signature motif of the structural class, all members of this superfamily also have a conserved Lysine involved in the catalytic reaction (Zharkov *et al*, 2003, 2002; Doublie *et al*, 2004). Fpg, Nei, NEIL2 and NEIL3 contain a conserved zinc (Zn) finger, while in NEIL1 this domain has been modified to a Zn-less finger domain (Doublie *et al*, 2004). Zn fingers are normally found to be involved in interactions to DNA, RNA or other proteins. NEIL3 is with its 67 kilodalton (kDa) remarkably larger than the other H2TH glycosylases. The C-terminal extension of NEIL3 contains a RAN binding protein (RANbp) - like Zn finger motif, a nuclear localization signal (NLS) and two additional Zn finger domains termed GRF domains after conserved residues in this motif (Bandaru *et al*, 2002; Morland *et al*, 2002). NEIL1 and NEIL2 are, like NTH1 and OGG1, bifunctional enzymes. However, while NTH1 and OGG1 carry out β-elimination to produce a single-strand break, NEIL1 and 2 possess β, δ-elimination activity (Morland *et al*, 2002; Hazra *et al*, 2002b; Dizdaroglu *et al*, 1999; Bjorâs *et al*, 1997). The catalytic mechanism of NEIL3 will be elucidated in the discussion of this thesis.

Dispensability and specialization of individual DNA glycosylases

Knockout (KO) mouse studies reveal that none of the five DNA glycosylases repairing oxidative lesions are essential for life (reviewed in Jacobs & Schär 2012). Because a broad selection of oxidized lesions is recognized by only five enzymes, the DNA glycosylases have a wide substrate range. All known oxidized lesions in DNA are recognized by more than one DNA glycosylase in vitro. Examples are FaPyG lesions which are recognized by NEIL1, NEIL3, NTH1 and OGG1 (Bjorâs *et al*, 1997; Luna *et al*, 2000; Morland *et al*, 2002; Hazra *et al*, 2002a) and the hydantoin lesions Sp and Gh that are processed by NEIL1-3 and NTH1 (Hailer *et al*, 2005a; Liu *et al*, 2010). The redundancy among the DNA glycosylases reflects the importance of efficient repair of oxidative damage, but in addition to compensate for each other, the DNA glycosylases tend to have more specialized functions in the organism. NTH1 and OGG1 are proposed to be the housekeeping DNA glycosylases responsible for global BER. They are ubiquitously expressed in most cells and tissues (Imai *et al*, 1998; Nishioka *et al*, 1999). Also, sequencing of the *OGG1* and *NTH1* promoter regions show nonappearances of typical TATA or CAAT boxes found in most genes under transcriptional control (Dhénaut *et al*, 2000; Imai *et al*, 1998). In a comparison study by Odell and coworkers, measurements of the cellular concentrations and the specific- and non-specific binding constants reveal that NTH1 is much better suited for global recognition and repair than NEIL1 (Odell *et al*, 2010). Moreover, NTH1 and OGG1 only recognize lesions in duplex DNA in contrast to the NEILs which seem to prefer single stranded lesions as well as bubble and fork structures, suggesting the NEILs to function during replication or transcription. NEIL1 interacts with proliferating cell nuclear antigen (PCNA), replication protein A (RPA), FEN1 and Werner's helicase (WRN), all components of the replication fork, indicating a role in replication associated repair (Dou *et al*, 2008; Theriot *et al*, 2010). NEIL2 is expressed independently of cell cycle, it associates to the RNA polymerase II and NEIL2 depleted cells accumulate more DNA damage in active than silent genes, suggesting NEIL2 to be involved in repair of transcribed genes (Hazra *et al*, 2002b; Banerjee *et al*, 2011; Neurauter *et al*, 2012). While NEIL1 and NEIL2 are expressed in most tissues throughout the organism, NEIL3 expression is not as prevalent.

Human NEIL3 is normally found expressed in thymus and testis (Morland *et al*, 2002). Also, elevated expression of NEIL3 in different types of cancers has been reported (Kauffmann *et*

al, 2008; Hildrestrand *et al*, 2009; Barry *et al*, 2011). Mouse Neil3 is expressed during embryonic development as well as in spleen, bone marrow, testis, thymus and distinct regions of brain harboring neural stem cells in adult animals (Torisu *et al*, 2005; Hildrestrand *et al*, 2009; Rolseth *et al*, 2008; Takao *et al*, 2009). Also, expression of human *NEIL3* mRNA is upregulated during S-phase of the cell cycle (Neurauter *et al*, 2012). The expression patterns of mNeil3 and hNEIL3 indicate a role for NEIL3 in proliferative tissue.

Neural stem cells

Stem cells are found in all multicellular organisms. They have the capacity to both self-renew and to differentiate into more specialized cells. Embryonic stem cells are derived from the inner cell mass of the blastocyst, they are pluripotent and can give rise to all cell types in the body. Somatic or adult stem cells are the multipotent stem cells found throughout the organism after embryonic development. These stem cells are responsible for replenishment of tissue throughout life (Brunt *et al*, 2012).

Neural stem cells (NSC) are the multipotent stem cells that can differentiate into the components of the central nervous system (CNS). NSCs can make commitments to neurogenesis and become functional neurons, or they can enter gliogenesis. Glia cells are the non-neural cells that support and protect the neurons. There are several types of glia cells present in the brain, among them are microglia, astrocytes and oligodendrocytes (figure 6). The neurons form networks and transmit information through chemical and electrical signaling. Neurogenesis is mainly happening during embryonic development and most of the neurons are as old as their host (Martino *et al*, 2011; Zhao *et al*, 2008). However, some neurogenesis continues in distinct parts of the adult brain: the subgranular zone (SGZ) of the hippocampus and the sub-ventricular zone (SVZ) of the lateral ventricles. Neurons differentiated in the SGZ migrate into the granule cell layer of dentate gyrus (DG) and become dentate granule cells. Neurons differentiated in the SVZ migrate through the rostral migratory stream to the olfactory bulb (Zhao *et al*, 2008; Spitzer, 2006).

20 % of the glia cells in the brain are microglia (Lawson *et al*, 1992), the macrophages of the CNS. The CNS has been considered an immune-privileged organ, lacking a lymphatic system and separated from the circulatory system by layers of endothelial cells known as the blood-

brain-barrier. Microglia protects the sensitive neural tissue from intruders through this barrier by phagocytosis (Yang *et al*, 2010; Tremblay *et al*, 2011). Astrocytes are the most abundant cell type of the human brain. These star shaped cells support the neurons physically through their structuring of the brain and biochemically through their provision of nutrients and ions. Upon brain injury, astrocytes play an important role in the repair and scaring process of the damaged tissue (Freeman, 2010; Sofroniew & Vinters, 2010). Oligodendrocytes coat and protect axons of the nerve cells with myelin sheets. The myelin is formed from the cell membrane and provides insulation to the axon, which increases the speed of the electrical impulses. A single oligodendrocyte can coat the axons of several nerve cells (Nave, 2010).

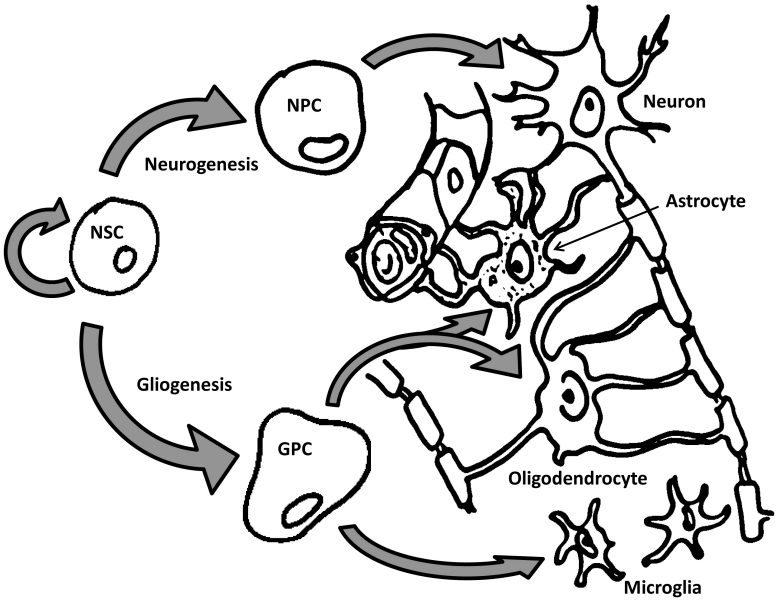


Figure 6. Cells of the brain. In the brain, neural stem cells (NSC) can self-renew or differentiate into neuronal linage, becoming functional neurons, or into a glial linage. Astrocytes, oligodendrocytes and microglia are all glial cells. NPC, neural progenitor cell; GPC, glial progenitor cell.

In adult humans, the brain consists of 2 % of the total body weight, but it consumes 10 % of the carbohydrates and almost half of the oxygen respired (Magistretti & Pellerin, 1996). The need for oxygen is high due to the vast amount of ATP used to maintain neuronal signaling. The high metabolic activity, low levels of antioxidant enzymes, high amounts of iron and the

formation of ROS during synthesis of neurotransmitters make the brain more prone to oxidative attack than other organs (Halliwell, 2006). The brain, as well as most other organs of the body consists of a diversity of cells with different needs regarding genome maintenance. NSCs are the most versatile cells of the brain and are put on high pressure to maintain genomic integrity (Rocha *et al*, 2012). A lesion in NSCs can, if left unrepaired, be fixated through replication, leading to mutations in a whole glial or neuronal linage. In terminally differentiated cells, like neurons, a certain degree of mutations are acceptable in the non-transcribed regions of the genome and global DNA repair is less active while the transcribed genes are repaired more efficient (Banerjee *et al*, 2011; Nouspikel, 2007; Nouspikel & Hanawalt, 2000, 2002). However, in contrast to most other post mitotic cells in the body, neurons are lasting throughout life.

Involvement of BER in aging and diseases

Accumulations of DNA damage are detected in several human pathologies including cancers, immunological dysfunctions and neurodegenerative diseases (Olinski *et al*, 2002; Hegde *et al*, 2012; Klaunig *et al*, 2011; Halliwell, 2006; Bohr *et al*, 2007). Defects in a DNA repair enzyme can be directly correlated to development of a disease, as previously outlined. However, the functions of the various BER glycosylases in disease prevention and progression are complex and there is little evidence about defects causing diseases.

Mouse models

Transgenic mouse models have been developed to unravel the function of the different BER components. Except Tdg-deficiency, that is embryonic lethal (Cortázar *et al*, 2011), mice deficient in one of the other DNA glycosylases display normal development and lack of an obvious phenotype. However, deeper investigations of the different mouse models are giving us interesting information about the role of the different DNA glycosylases in modulating disease risk. In contrast to the mild phenotypes of the DNA glycosylase deficient mice, the downstream BER enzymes Ape1, Pol β, Lig III and Xrcc1 are all lethal (Ludwig *et al*, 1998;

Sugo *et al*, 2000; Puebla-Osorio *et al*, 2006; Tebbs *et al*, 1999). Following is a summary of what is known about mice lacking oxidative DNA glycosylases.

Ogg1-deficient mice accumulate oxidative 8-oxoG lesions followed by elevated mutation frequency in liver (Klungland *et al*, 1999; Minowa *et al*, 2000). Aged mice lacking Ogg1 exhibit spontaneous motor behavior deficiency as well as neurochemical and histological changes in the brain (Cardozo-pelaez *et al*, 2012). Mutyh-deficient mice do also accumulate 8-oxoG in liver, but display no overt phenotype. However, *Ogg1*$^{-/-}$*/Mutyh*$^{-/-}$ mice is predisposed for developing lymphoma and tumors in lungs, ovaries, and small intestines (Russo *et al*, 2004; Xie *et al*, 2004). Nth1-deficient mice accumulates FaPyA in kidney and liver (Chan *et al*, 2009). Moreover, *Nth1*$^{-/-}$*/Ogg1*$^{-/-}$ mice showed impaired repair capacity of 5-OHC and 5-OHU in mitochondria (Karahalil *et al*, 2003). Neil1-deficient mice accumulate FaPyA and FaPyG lesions in brain, kidney and liver (Chan *et al*, 2009), and they exhibit a high chance of developing obesity followed by symptoms similar to human metabolic syndrome (Vartanian *et al*, 2006). The increased susceptibility to overweight is suggested to be caused by the low tolerance to oxidative stress (Sampath *et al*, 2011). It is worth mentioning that also Ogg1-deficient mice were a bit larger compared to their wild-type littermates (Arai *et al*, 2006). *Nth1*$^{-/-}$*/Neil1*$^{-/-}$ mice showed a higher incidence of tumors on lungs and liver (Chan *et al*, 2009). There are so far no reports about the consequence of Neil2 deficit, but we are breeding them in the lab and they are viable and fertile (personal communication with Gunn Hildrestrand). The Neil3-deficient mouse model was published some years ago, where no phenotype was detected (Torisu *et al*, 2005). Characterization of the Neil3-deficient mouse model is included in this thesis and will be discussed later.

Polymorphism and disease

Single nucleotide polymorphisms (SNPs) of DNA glycosylases may affect BER efficiency. A third of the human population has the OGG1 SNP variant Ser326Cys. Biochemical analysis of OGG1Ser326Cys reveals a decrease in glycosylase activity (Audebert *et al*, 2000; Sidorenko *et al*, 2009). A large number of studies have investigated whether this polymorphism leads to predisposition for different cancers, where most studies find none or marginally correlation with disease (Weiss *et al*, 2005; Li *et al*, 2008). Patients with biallelic

deficiency in the *MUTYH* gene have higher susceptibility to develop MUTYH-associated polyposis (MAP) a common type of colorectal cancer (Al-Tassan *et al*, 2002; Jones *et al*, 2002; Jasperson *et al*, 2010). MAP has also been found to be associated with tumors on breasts, ovarian, bladder and skin (Vogt *et al*, 2009). No convincing correlation between variants of the NEIL enzymes and disease has been found. SNPs of the NEILs have been reported in cancer patients, but most of these variants are only found in a small group of patients (usually one to two), or they have also been found in healthy individuals (Dallosso *et al*, 2008; Forsbring *et al*, 2009). An example is the NEIL2 variants Arg103Gln and Arg257Leu, each found in a patient with family history of gastric cancer, and not in the control group in one study (Broderick *et al*, 2006). Another study identifies the same variants in both cancer patients and in the control group (Dallosso *et al*, 2008). There are no reports of NTH1 variants associated with disease.

Aging and neurodegeneration

The 'oxidative stress theory of aging' suggests that accumulation of oxidative lesions in the genome during the life span of an organism results in a gradual decline of cellular functions which eventually leads to death (Harman, 1981). The risk for developing a neurodegenerative disease increases with age. Accumulations of oxidative damage are detected in various neurodegenerative conditions like Parkinson`s disease (PD), Alzheimer`s disease (AD), Huntington`s disease (HD) and amyotrophic lateral sclerosis (ALS). There is growing evidence supporting the fact that BER is involved in maintenance and protection of neuronal tissue, but no clear correlation between BER deficiency and neurodegenerative diseases has been demonstrated (Hegde *et al*, 2012; Halliwell, 2006; Coppedè & Migliore, 2009).

Several reports suggest that OGG1 functions in protecting neurons against neurodegeneration. The OGG1 polymorphic variant Ser326Cys has been found to be weekly associated with ALS and HD, but not with AD and HD (Coppedè & Migliore, 2009; Coppedè *et al*, 2010, 2007a, 2007b). Another study finds that four out of 14 AD patients had OGG1 variations, two single base deletions resulting in abolished OGG1 activity, and two single base substitutions resulting in reduced OGG1 activity, while none variations were detected in the control group (Mao *et al*, 2007). Ischemic stroke is a neurodegenerative event caused by

blocked blood supply to parts of the brain. The oxygen deprivation leads to mitochondria dysfunction and overproduction of ROS, immediately followed by extensive oxidative DNA damage (Saeed *et al*, 2007; Vieira *et al*, 2011). Stroke induces expression of several BER enzymes, including Ogg1, Neil1, Ung, Xrcc1, Polβ, Ape1 and LigIII in mice and rats (Lan *et al*, 2003; Li *et al*, 2011; Canugovi *et al*, 2012; Endres *et al*, 2004; Liu *et al*, 2011). Hypoxia-ischemia (HI) is a method mimicking stroke in animal models. Transgenic mice, deficient in Neil1, Ung or Ogg1 do all get larger brain damage after HI compared to wild-type mice, demonstrating an important role for these glycosylases in repair of stress induced damage (Canugovi *et al*, 2012; Liu *et al*, 2011; Endres *et al*, 2004). The DNA glycosylases Neil1, Neil2, Nth1 and Ogg1 are expressed in neuronal tissue in mouse brain throughout development and adulthood (Rolseth *et al*, 2008), while Neil3 is only found during development as well as in SVZ and DG, the distinct areas of the adult brain harboring NSCs (Hildrestrand *et al*, 2009). These findings suggest a role of Neil3 in maintenance of NSCs.

PRESENT INVESTIGATION

Aims of the study

DNA is continuously modified by ROS from the cellular environment (Hegde *et al*, 2012). BER is the major pathway for repair of ROS induced damage, and is triggered by the action of a DNA glycosylase, which recognizes and excises the damaged nucleotide (Dalhus *et al*, 2009). There are three homologues of endonuclease VIII-like (NEIL) DNA glycosylases in mammalian cells; NEIL1, NEIL2 and NEIL3. At the time this project was initiated, little was known about NEIL3.We hypothesized that NEIL3 was a functional DNA glycosylase, recognizing oxidative lesions in DNA. The aim of the first part of the study was to characterize the biochemical properties of recombinant human NEIL3. To address this, we cloned *Neil3* into a suitable expression vector and developed a purification strategy for the human NEIL3 protein (paper I). Further, we performed site-directed mutagenesis and characterized substrate specificity and reaction mechanism by *in vitro* excision/incision assays of wild-type and mutant enzymes to pinpoint residues involved in catalysis (paper II). These experiments would give us knowledge about the catalytic properties of the recombinant enzyme, which makes the fundament for further research on NEIL3.

The most prominent expression of mouse Neil3 is found in the developing brain and in compartments of the adult brain harboring NSCs (Hildrestrand *et al*, 2009) as well as in

proliferative lymphatic tissue (Torisu *et al*, 2005). The *Neil3* knockout mice are viable and fertile, lacking an overt phenotype (Torisu *et al*, 2005). Recombinant mouse Neil3 is found to recognize and repair the hydantoin lesions Sp and Gh, with preference for lesions in ssDNA (Liu *et al*, 2010). Sp and Gh have a high mutagenic potential and are strong replication blocks *in vivo* (Henderson *et al*, 2003). We hypothesize that Neil3, by the removal of hydantoin lesions from DNA, has a role in maintaining genome integrity and thus promoting proliferation of neural and lymphatic tissue. The aim of the second part of this thesis was to establish the role of Neil3 in proliferative tissues. To address this, we developed a Neil3-deficient mouse model and investigated brain structure and repair activity of different tissues in comparison to Neil3-proficient mice (paper III). Further, we exposed wild-type and Neil3-deficient mice to hypoxia-ischemia (HI), a method known to induce extensive DNA damage and apoptosis in brain, followed by tissue regeneration (paper IV). These studies would give us knowledge about the biological role of Neil3 in maintaining genome integrity of stem cells.

Summary of results

Paper I: Expression and purification of NEIL3, a human DNA glycosylase homolog.

NEIL3 shares high sequence similarities to NEIL1 and NEIL2, but has a long C-terminal extension with a unique GRF Zn-finger domain. The biochemical properties of NEIL3 were, at the time-point of this study, not elucidated. Here, we present a protocol for successful expression and purification of a truncated human NEIL3 (amino acids 1-301) that contain the complete *E. coli* Fpg/Nei-like glycosylase domain (GD) but lack the C-terminal. The full-length (FL) NEIL3 was also expressed in this study, but we were only able to partially purify it. Bioinformatics analysis of NEIL3 predicts that a ~100 residues segment, between the GD and the GRF Zn-finger is disordered. Disordered regions in proteins do usually not have a tertiary structure and often lead to difficulties in protein expression, purification and crystallization. Removal of the disordered segment resulted in successful expression and purification of GD NEIL3. However, we did not detect any glycosylase activity on the substrates tested. We suggest that the disordered segment of NEIL3 requires specific conditions only found in the eukaryotic cell, like posttranslational modifications (PTMs) or chaperones, to obtain correct tertiary structure.

Paper II: **Human NEIL3 is mainly a monofunctional DNA glycosylase with affinity to spiroiminodihydantoin and guanidinohydantoin.**

The main substrates of NEIL3 have been reported to be the hydantoins Sp and Gh, Tg and FaPy lesions (Liu *et al*, 2010, 2012). In this work, we have purified the core glycosylase domain (GD) of human NEIL3 and elucidated the catalytic mechanisms. GD NEIL3 removed the hydantoin lesions Sp and Gh in single- and double-stranded DNA as well as 5-OHC and 5-OHU lesions in ssDNA. In contrast to the concerted catalytic mechanisms of NEIL1 and NEIL2, the DNA glycosylase/AP lyase activities of NEIL3 were demonstrated to be non-concerted, where base release was more efficient than strand incision. Fpg/Nei and NEIL1/2 contain a catalytic Pro2, while this amino acid is replaced by Val in NEIL3. Biochemical mapping of the catalytic N-terminus of NEIL3, by site-directed mutagenesis, demonstrated that Val2 is the residue responsible for the non-concerted action of NEIL3. Furthermore, Lys81 is found to be the catalytic residue, essential for DNA glycosylase activity. We suggest that the substitution of the second amino acid from proline to valine has directed NEIL3 to act as a monofunctional glycosylase in *vivo*.

Paper III: Loss of Neil3, the major DNA glycosylase activity for removal of hydantoins in single-stranded DNA, reduces cellular proliferation and sensitizes cells to genotoxic stress.

Neil3 is expressed in brain during embryonic development. We analyzed and compared brain structure of newborn wild-type and Neil3-deficient mice by immunoreactivity, but did not find any differences regarding brain architecture or distribution of neural and glial tissue. However, *in vitro* culturing of neuronal stem/progenitor cells revealed that Neil3-deficient cells had unaffected differentiation capacity, but impaired ability to self-renew. Proliferation was also reduced in mouse embryonic fibroblasts (MEFs) derived from Neil3-deficient embryos and these cells were sensitive to both the oxidative agent paraquat and interstrand cross-link inducing agent cisplatin. Total cell extract of brain, heart, thymus and spleen from Neil3-deficient mice displayed reduced repair activity against Sp and Gh lesion in ssDNA, assayed by excision/incision assays. Our data suggests that Neil3 is involved in repair of hydantoin lesions in proliferative tissue.

Paper IV: Endonuclease VIII-like 3 (Neil3) DNA glycosylase promotes neurogenesis induced by hypoxia-ischemia.

To address our hypothesis that Neil3 is involved in DNA repair of NSPCs, we exposed wild-type and Neil3-deficient mice to oxidative stress by HI. HI resulted in major tissue damage in the forebrain. We did not detect differences in neuronal death between wild-type and Neil3-deficient animals three to ten days after HI. However, six weeks after injury, the Neil3-deficient mice demonstrated impaired recovery of brain tissue by reduced volume of neuronal tissue. Supporting these results, Neil3-deficient animals showed a reduction in proliferating NSPCs migrating to the striatum three to ten days after HI, compared to wild-type. Neil3-deficient animals also showed a decreased microglial response to HI. *In vitro* cultured NSPCs (neurosphere culture), isolated from wild-type animals after HI, showed a high proliferative response which was absent in the Neil3-deficient cells. Also, the differentiation pattern of the Neil3-deficient neurospheres was altered with fewer cells entering neuronal linage. Further, the Neil3-deficient cells exhibited increased accumulation of strand breaks compared to wild-type cells. Neil3-deficient neurospheres had impaired repair activity towards Sp and Gh lesions in ssDNA as measured by excision/incision assay of total cell extract. Our data supports a role for Neil3 in promoting proliferation of NSPCs by removal of the replication blocks; Sp and Gh.

DISCUSSION

Structural properties of NEIL3

After the discovery of the base excision mechanism, BER was believed to be one of the simpler repair pathways in the cell, involving four to five enzymes only (Dianov & Lindahl, 1994). But along with new evidence, it is getting clearer that BER is highly complex, involving a network of extensively regulated sub-pathways, involving more accessory proteins, transcription factors and posttranslational modifications (PTMs) than first assumed (Sampath *et al*, 2012; Jacobs & Schär, 2012; Odell *et al*, 2012; Wallace *et al*, 2012). Paper I and II are studies on the structural properties of NEIL3 and here I discuss my findings in accordance to its presumed functions in BER.

Reaction mechanism

Along with the discovery of the NEIL enzymes in 2002, a new sub-pathway of BER was identified. The DNA glycosylases specific for oxidative base lesions are all bifunctional with intrinsic AP lyase activity. OGG1 and NTH1 catalyze β-elimination, generating 3'PUA

which is further processed by APE1, while NEIL1 and NEIL2 perform β,δ-elimination resulting in 3'P which is processed by PNK (Wiederhold *et al*, 2004). Involvement of PNK in repair of oxidative damage has been suggested before, however the direct role of PNK in BER was not clear before the discovery of the NEILs (Karimi-Busheri *et al*, 1999; Yang *et al*, 1996; Rasouli-Nia *et al*, 2004). It is now believed that NEIL1/NEIL2 together with PNK act in an APE1 independent subpathway of BER (Wiederhold *et al*, 2004). Interestingly, in contrast to the other NEIL enzymes, NEIL3has uncoupled excision/incision activities where base excision is more efficient than strand incision. Further, NEIL3 incise the damaged strand mainly by β-elimination, although some of the abasic DNA is processed by β,δ-elimination (paper II, Liu et al. 2010). In paper II we show that substituting Val2 in NEIL3 to Pro, the catalytic nucleophile in NEIL1 and NEIL2, results in an enzyme with concerted DNA glycosylase/AP lyase mechanism performed by β,δ-elimination.

These data indicates that Val2 is the residue responsible for the monofunctional reaction mode of NEIL3. Moreover, a NEIL2 homologue found in mimivirus (mvNeil2) also contains a valine as the second amino acid and incises DNA by a mix of β- and β,δ-elimination (Bandaru *et al*, 2007). Further, studies on Fpg/Nei enzymes have shown that substitution of Pro2 affects the reaction mechanism, supporting our results (Saparbaev *et al*, 2002; Burgess *et al*, 2002; Zharkov *et al*, 1997; Vik *et al*, 2012).

Uncoupled glycosylase and AP lyase activities have also been shown for mammalian OGG1 and NTH1 (Morland *et al*, 2005; Marenstein *et al*, 2001). This suggests that the intrinsic AP lyase activity of the DNA glycosylases plays a more prominent role in the PNK dependent than the APE1 dependent subpathway of BER. APE1 has a remarkably higher catalytic turnover rate on AP sites than NEIL3 (Strauss *et al*, 1997) and we suggest that the AP lyase activity of NEIL3 is not of biological significance in mammalian BER. The *E. coli* enzymes Fpg, Nth and Nei have tightly coupled glycosylase/AP lyase activities, catalyzing strand incision at about the same rate as base release (Krokan *et al*, 1997; Kow & Wallace, 1987). The dominant monofunctional reaction mode of NEIL3 indicates evolutionary pressure/adaptation to avoid strand incision coupled to the base excision reaction, supporting that NEIL3 recruits APE1 to perform the incision reaction at the abasic site. NTH1 and OGG1 are reported to repair oxidative lesions in duplex DNA only, while NEIL1, NEIL2 and NEIL3 also recognize and repair lesions in single-stranded DNA (Paper II, Hong Dou et al.

2003; D O Zharkov et al. 2000). Therefore, NEIL3 appears to be the only DNA glycosylase removing hydantoin- and FaPy lesions in ssDNA without generating a strand break.

Another feature unique for NEIL3 is a 30 residue insert found in the glycosylase domain of the protein (paper II). This loop is positioned close to the lesion recognition pocket, it is not conserved and varies in length and sequence among species. The function is unknown and we speculate that it is involved in regulating the enzyme by blocking/promoting DNA glycosylase activity.

Benefits of a disordered C-terminal

Disordered protein segments do not form a stable tertiary structure *in vitro*. Most of the mammalian DNA glycosylases contain a protein sequence, 50-100 amino acids in size, with a high degree of disorder. OGG1 is among the exceptions and have shorter (about 10 residues) disordered fragments in both ends. NEIL1 contains a 100 amino acid long disordered sequence in the C-terminus, NEIL2 contains an internal disordered segment at the residues 45-130 and NEIL3 contains a disordered region of proximately 100 residues in the C-terminal prior to the GRF Zn finger (Paper I, Hegde *et al*, 2010). In contrast, disordered regions are absent in the *E.coli* Nei homologue (Hegde *et al*, 2010). Interestingly, removal of 56 C-terminal residues of human NEIL1 leads to a fourfold increase in glycosylase activity (Doublie *et al*, 2004). Similarly, human NTH1 has an N-terminal extension lacking in *E.coli* Nth, and truncation of these residues leads to increased activity (Liu & Roy, 2002). In agreement with these results, we find that full-length NEIL3 has weaker glycosylase activity on Sp and Gh lesions in single- and double-stranded DNA than NEIL3 with the C-terminal truncation (GD NEIL3) (unpublished data). In partial agreement with our unpublished results, Liu and coworkers demonstrates that recombinant mouse Neil3 get enhanced activity against hydantoin lesions in dsDNA but not ssDNA by truncation of the disordered C-terminal segment (Liu *et al*, 2010). We propose that the disordered segment of NEIL3 regulates the enzymatic activity, by inhibiting activity in the absence of downstream BER components. The presence of non-conserved, disordered regions in all DNA glycosylases repairing oxidative lesions, suggests that these segments have important functions like DNA and protein interactions, damage recognition or protein regulation.

DNA glycosylases search for base lesions by a combination of sliding along the helix and intersegmental transfer (Friedman & Stivers, 2010; Odell *et al*, 2012). Proteins with the H2TH DNA binding domain (homeodomain proteins) are reported to have increased number of contact points to DNA through disordered segments (Tóth-Petróczy *et al*, 2009) and they are suggested to efficiently jump from one DNA molecule to another through an intermediate, where the recognition pocket of the protein is interacting with one DNA fragment and the disordered tail is interacting with a second (Vuzman *et al*, 2010). Tóth-Petróczy and coworkers also claim that homeodomain proteins fold correctly only when their disordered tails are in contact with DNA.

It is established that the proportion of disordered proteins correlates with the complexity of an organism (Ward *et al*, 2004). Genome wide analysis have also shown that proteins that interact with many partners are more likely to have disordered sections than proteins with few partners (Haynes *et al*, 2006). NEIL1 forms complexes with the downstream BER proteins FEN1, POL β, LIG III, XRCC1 and PCNA through interactions with the 100 residues long disordered C-terminal (Hegde *et al*, 2008b; Wiederhold *et al*, 2004; Dou *et al*, 2008). NEIL3 is found to co-localize with replication protein A (RPA) (Morland *et al*, 2002). Further, pull-down experiments with recombinant NEIL3 C-terminal in fusion with a GST tag in cell extract indicate interaction with poly[ADP-ribose] polymerase 1 (PARP1), Ku70, DNA protein kinase (DNA-PK) and RPA (own unpublished results). PARP1 is known to be involved in single-strand break repair. PARP1, Ku70 and DNA-PK are central proteins involved in double-strand break repair, while RPA binds single-stranded DNA during repair and replication. RPA is also involved in homolog recombination and telomere maintenance (Wang *et al*, 2012; Muller *et al*, 2005; Pawelczak *et al*, 2011; Sakaguchi *et al*, 2009). NEIL3, with the long disordered C-terminal, is likely to interact with several other proteins.

PTMs of proteins play important roles in many cellular processes including DNA repair. PTMs on DNA glycosylases may regulate substrate binding, subcellular localization and protein-protein interactions (Fan & Wilson, 2005). Acetylation of Lys49, in the disordered region of NEIL2, is a switch which inactivates the DNA glycosylase/AP lyase activity (Bhakat *et al*, 2004). Acetylation of Lys338 and Lys341 in the short disordered C-terminal of OGG1 increases the turnover for 8-oxoG lesions by reducing the affinity for AP sites (Bhakat *et al*, 2006). There is a lot to be unraveled about PTMs on NEIL3. A single modification can

be essential for proper repair activity, but also for correct folding (Tokmakov *et al*, 2012). Types and positions for modifications can vary in between eukaryotic and prokaryotic cells (Soppa, 2010). In paper I and II, active fractions of GD NEIL3 without the disordered C-terminal were easily purified from *E. coli,* while FL NEIL3 tended to form aggregates and displayed irregular migration during separation on a denaturing gel. On the contrary, by expression in a mammalian system with rabbit reticulocyte lysate, FL NEIL3 migrated as predicted by size (paper I). The lack of correct PTMs of FL NEIL3 expressed in *E. coli* is one possible explanation for the aberrant tertiary structure.

A unique role for Neil3 in proliferative tissue

We generated a mouse model deficient in the *Neil3* gene by engineered depletion of exon 3-5 to unravel the *in vivo* functions of Neil3 (paper III and IV). In accordance with a previous report (Torisu *et al*, 2005), our Neil3-deficient mice were healthy and fertile with no profound phenotype.

Neil3 in neural stem cells

In paper IV, we exposed perinatal mice to hypoxia and ischemia (HI), a well-known method mimicking hypoxic-ischemic encephalopathy, a type of stroke, in the perinatal brain. HI resulted in major brain damage, with no differences in infarct size, between Neil3-proficent and Neil3-deficient mice, when analyzed three to ten days after injury. However, six weeks after injury, Neil3-deficient mice demonstrated impaired recovery of brain tissue, suggesting a role for Neil3 in neuronal stem/progenitor cells (NSPCs) rather than post mitotic brain tissue.

NSPCs are limited in number in mouse brain, and had to be propagated *in vitro* for further analysis. There is currently no method for homogenous culturing of neural stem cells. Cells from the SVZ form floating sphere-like structures, neurospheres, when propagated in the right conditions (Ahmed, 2009; Arvidsson *et al*, 2002; Jensen & Parmar, 2006; Reynolds & Weiss, 1992). Neurospheres consists of a mix of stem cells, progenitor cells and terminally differentiated cells, where the ability to form new neurospheres (colony forming unit, CFU)

in the next passage is correlated with the amount of stem cells in the population (Reynolds & Weiss, 1992). As demonstrated in paper III and IV, neurospheres from the Neil3-deficient mouse showed reduced proliferative capacity. Moreover, the differentiation pattern of the Neil3-deficient neurospheres, isolated after HI, was altered with less cells entering neuronal linage (paper IV). These results are in partly agreement with another report where knockdown of Neil3 resulted in decreased proliferation, gliogenesis and neurogenesis of NSPCs isolated from the embryonic rat brain (Reis & Hermanson, 2012). Neil3-deficient neurospheres accumulated DSBs after exposure to paraquat, while we did not find a difference between the two genotypes in accumulation of DSBs in the ischemic brain tissue. In addition, we demonstrate that wild-type, but not Neil3-deficient neurospheres were able to repair Sp and Gh lesions from ssDNA (paper IV), while the repair activity against these lesions were almost undetectable in whole-brain extract from both genotypes (paper III). Altogether, our results suggest that Neil3 does not function in the terminally differentiated cells of the brain, but have a prominent role in NSPCs. Ten days after injury, we detected a decreased number of proliferating NSPCs migrating from SVZ to repopulate the striatum in Neil3-deficient mice, compared to wild-type mice. The pool of quiescent NSPCs in SVZ was not affected by Neil3-deficiency, suggesting Neil3 to have a role in proliferative, but not quiescent NSPCs. Other groups have performed transient middle cerebral artery occlusion on adult mice deficient in Neil1, Ogg1 and Ung, and in contrast to our Neil3-deficient model these mice showed increased tissue damage due to impaired repair of stress induced DNA damage in postmitotic brain tissue (Liu *et al*, 2011; Endres *et al*, 2004; Canugovi *et al*, 2012).

Oxygen is normally tightly controlled within the brain (Simon & Keith, 2008; Vieira *et al*, 2011). Various reports have listed oxygen concentrations in rodent brain to range from 0.1-5 % depending on the specific regions (Seyde & Longnecker, 1986; Sick *et al*, 1982). Oxygen is known to be involved in control of cellular fate of NSPCs. Low level of oxygen in the embryonic brain stimulates hypoxia-inducible factor 1α (HIF-1α) which promotes proliferation and is essential for early brain development and neurogenesis (Iyer *et al*, 1998). Increasing oxygen tension degrades HIF-1α which promotes differentiation (Mazumdar *et al*, 2010). When NSPCs are dissected out from their natural niche and grown *in vitro* in ambient air (21 % oxygen) in an incubator, they are exposed to non-physiological levels of oxygen. NSPCs derived from Neil3-deficient mice display impaired proliferation while we do not find

any aberrations in the cellular composition of the brain of newborn KO animals. The disagreement in data may be due to the hyperoxic conditions *in vitro*.

A high level of ROS induced damage is, as outlined previously, associated with neurodegenerative diseases and aging (Hegde *et al*, 2012; Halliwell, 2006; Jeppesen *et al*, 2011). Interestingly, reactive oxygen is not always a cellular threat. There is an emerging understanding of ROS functioning as messenger molecules in redox signaling pathways controlling cellular physiology including apoptosis, proliferation and differentiation of embryonic and somatic stem cells (Nitti *et al*, 2010; Varum *et al*, 2009; Sauer *et al*, 2000; Le Belle *et al*, 2011; Tsatmali *et al*, 2006). Elevated levels of ROS are associated with stem cell differentiation through activation of the p38 mitogen-activated protein kinase (p38 MAPK) (Cho *et al*, 2006; Ito *et al*, 2006; Le Belle *et al*, 2011). Moreover, research on naïve neurospheres has demonstrated that stress-induced accumulation of mitochondrial DNA damage leads to a shift in the differentiation of NSCs toward astrocytic linage (Wang *et al*, 2011, 2010). These studies underscore the importance of DNA glycosylases to promote correct proliferation and differentiation of NSPCs.

Recently, we published a report about impaired neurogenesis of aged Neil3 knockout mice, and consequences for learning and memory (Regnell *et al*, 2012). Behavioral studies revealed that Neil3-deficient mice have learning and memory deficits and a reduced anxiety-like behavior. Similar to the results from paper IV, NSPC from aged Neil3 knockout mice showed impaired proliferative capacity and reduced repair of hydantoins in ssDNA. In addition, we here demonstrated that aged Neil3-deficient mice have aberrant distribution of excitatory and inhibitory receptor subunits and altered synapse composition within hippocampus. It appears that Neil3 is required for maintenance of adult neurogenesis to counteract the age-associated reduction of cognitive performance.

Neil3 in lymphatic organs

Mouse *Neil3* mRNA is expressed in thymus and spleen, and repair of hydantoin lesions in ssDNA is impaired in Neil3-deficient extracts from these organs (paper III), suggesting a functional role for Neil3 in hematopoietic/lymphoid organs. In support of this hypothesis, mouse Neil3 has previously been reported to be expressed in bone marrow and several B-cell

lines, and expression is induced by mitogen stimulation where splenocytes starts to proliferate (Torisu *et al*, 2005). The Neil3-deficient animals generated by Torisu and coworkers also exhibited a tendency of reduction of peripheral white blood cell number. AP endonuclease 2 (Ape2) shares several features with Neil3 and there are several reports on the mammalian Ape2 and its function in lymphoid tissue (Ide *et al*, 2004; Sabouri *et al*, 2009; Guikema *et al*, 2011). Ape2 and Neil3 are structurally related, both contain a long stretch of disordered structure and a duplicate of the GRF Zn-finger at the C-terminal. The GRF Zn-finger is a unique motif, only found in a very few other mammalian proteins, suggesting Neil3 and Ape2 to be involved in the same pathway where they share common interaction partners. Further, they both have reduced AP lyase functions compared to their homologues Neil1/2 and Ape1 (paper II, Hadi & Wilson, 2000; M Takao et al., 2009). Both proteins have induced expression in S-phase, Neil3 has a putative PCNA binding motif and Ape2 has been shown to associate with PCNA, linking their activities to replication (Neurauter *et al*, 2012; Morland *et al*, 2002; Burkovics *et al*, 2009; Ide *et al*, 2004). Ape2 is found to be necessary for normal cell cycle progression in proliferating B cells and plays an important role in recovery of lymphoid tissue cells after chemotherapeutic depletion of the bone marrow (Guikema *et al*, 2011; Ide *et al*, 2004). Based on our knowledge about the importance of Neil3 in proliferation of NSPCs, we suggest that Neil3 and Ape2 have similar functions in maintaining DNA integrity in proliferating stem/progenitor cells.

Consequences of hydantoin lesions in the genome

Little is known about the magnitude the Sp and Gh lesions in the mammalian genome. Hydantoins are formed after further oxidation of 8-oxoG, which has been shown to be 1000 times more reactive against peroxynitrite than guanine itself (Helbock *et al*, 1998; Uppu *et al*, 1996). The first report of detection of these lesions in the mammalian genome was published a few months ago: Sp and Gh levels were correlated to the level of 8-oxoG, and detected with a frequency of one to seven lesions per 10^8 nucleotides in mouse colon and liver, almost 100 times less frequent than the parental 8-oxoG lesion (Mangerich *et al*, 2012). While 8-oxoG is mildly mutagenic, leading to a G → T mutation with a frequency of less than 10 % if not repaired, Sp and Gh will always result in G → T or G → C mutation if left unrepaired prior to replication (Duarte *et al*, 1999; Klein *et al*, 1992; Cheng *et al*, 1992). Gh and Sp lesions

have shown, in contrast to 8-oxoG, to be strong replication blocks *in vivo,* where lesion bypass efficiency is sequence dependent (Henderson *et al*, 2002; Delaney *et al*, 2007; Henderson *et al*, 2003). Nei-deficient *E. coli* accumulates 20-fold more Sp lesions than wild-type during oxidative stress (Hailer *et al*, 2005b).

In paper III and IV we demonstrated by *in vitro* experiments that repair of hydantoin lesions in ssDNA in mouse NSPCs, thymus and spleen is mainly performed by Neil3. Even though the repair activity on hydantoin lesions in ssDNA was just above detection level, we detected a clear correlation between excision/incision activity and expression of Neil3 (paper III). Repair activities were in general higher for hydantoin lesions in dsDNA than ssDNA, which can be explained by the presence of single-strand DNA-binding proteins (SSB) and other proteins with affinity for ssDNA in the cell extract. SSB are abundant in the cell and can cover the ssDNA substrate, making it inaccessible for recognition and excision by the DNA glycosylase in the *in vitro* assay. Adding titrated competitor ssDNA to the reaction was essential to detect activity, but the reaction conditions might still not be optimal. Nevertheless, the DNA exists as a duplex in most contexts, and likely, the bulk of the DNA glycosylases will be engaged in the repair of lesions in dsDNA.

Sp and Gh lesions in DNA are recognized and repaired by Nth1 and Neil1-3 in mammals, while only the Neils are reported to recognize hydantoins in ssDNA (Hailer *et al*, 2005a; Liu *et al*, 2010). Neil1 repairs Sp and Gh lesions 100-fold more efficient than its previously reported standard substrates Tg and 5-OHC (Krishnamurthy *et al*, 2008). The absence of repair activity of hydantoin lesions in ssDNA in NSPCs, thymus and spleen from Neil3-deficient mice suggest either that expression of Neil1 and Neil2 is too low for efficient catalysis, or Neil1 and Neil2 are present, but limited to other functions or substrates in proliferative tissue.

Conclusion

The `loss` of the active site residue Pro2 in human NEIL3 provides different catalytic properties than NEIL1 and NEIL2. Incision of DNA when it is in a single-stranded context may result in fatal strand breaks. We speculate that NEIL3 is a monofunctional glycosylase *in vivo*, recruiting downstream BER enzymes to complete the repair.

In this study, we have shown that Neil3 is a DNA glycosylase important for proliferating cells. We suggest that Neil3 removes hydantoin lesions in proliferating cells to prevent blockage of replication, transcription, or both. We also hypothesize that Neil3 may be involved in region specific repair, for instance in promoters of genes involved in proliferation and/or differentiation.

CONCLUDING REMARKS

When I started working with NEIL3 some years back, there were only a few published reports. We cloned the sequence and had a wish to express and purify the protein. NEIL3 has been challenging to work with. The recombinant protein formed aggregates, overexpression in eukaryotic cell lines was toxic, while endogenous expression in most cell types was below detection level. I guess I have experienced the same feeling of hopelessness as most PhD students do. It feels good to look back on the puzzle where we, together with many other groups have contributed to find the pieces. There is still much to be unraveled about NEIL3. When the NEILs were discovered, they were first suggested to be back up enzymes for OGG1 and NTH1. With new evidence, like the lack of cancer phenotypes in DNA glycosylase deficient mice strands, we speculate that BER is not simply repair, but a finely tuned system for regulation of DNA base modifications in specific contexts to maintain cell properties and tissue homeostasis, i.e. maintenance of epigenetic state. BER is highly regulated and all the contributors probably have their unique function. Factors like cell type, cell cycle stage, cell maturity, proliferation/differentiation stage, tissue type, chromatin state

as well as extent, type, and location of lesions will likely influence on the choice of enzymes and repair pathways. Repair of oxidative damage is of vital importance for the organism, and the DNA glycosylases have evolved to be flexible so they can step in for each other if one is defect as well as during massive ROS induction.

Our plans for future experiments are many. We have been trying to crystallize the glycosylase domain of human NEIL3 for years without success. A crystal structure can give us important information about the architecture of the active site and the base recognition pocket with the 30 nucleotide non-conserved loop, unique for NEIL3. The recent evidence of the presence of Sp and Gh lesions in mammalian tissue supports that base excision indeed may be the canonical function of Neil3. As a continuance of paper IV, it would be valuable if we were able to detect Sp and Gh lesions in brain tissue and neurospheres from Neil3-deficient mice. Further, we wish to expose *Neil3*$^{-/-}$*/Ogg1*$^{-/-}$ mice to HI. We predict these mice to accumulate even greater amount of hydantoins, due to their reduced capacity of 8-oxoG removal. Implementing detection of Sp and Gh in the genome, as well as a mouse model with a more pronounced phenotype will be useful tools in our search for the mechanisms leading to impairment in proliferation.

After my dissertation, I will start working on another interesting feature of NEIL3: Its involvement during Human Immunodeficiency virus (HIV) infection. This will be collaboration with Stephen Goff's lab at Columbia University. NEIL3 has been suggested to be a host factor for HIV, after it was picked up in a genome wide siRNA screen in 2008 (Zhou *et al*, 2008). Later, also other components of BER, including OGG1, NEIL1, NTH1 and POL β, have been implicated a role in the viral life cycle (Espeseth *et al*, 2011; Yoder *et al*, 2011). *NEIL3* mRNA is strongly upregulated in CD4^{+} cells of HIV patients, and its expression correlates with virus count (personal communication with Pål Aukrust, Rikshospitalet). Further, we have transduced wild-type and Neil3-deficient MEF cells with a lentiviral vector system, derived from HIV, and found that Neil3-deficient cells have a magnitude lover transduction rate than the wild-type cells (own unpublished results). With our knowledge on NEIL3 and BER, and with the Goff labs expertise on HIV, we hope to get an insight into the use of DNA glycosylases in the lentivirus life cycle

REFERENCES

Ahmad R, Rasheed Z & Ahsan H (2009) Biochemical and cellular toxicology of peroxynitrite: implications in cell death and autoimmune phenomenon. *Immunopharmacology and immunotoxicology* **31:** 388–96

Ahmed S (2009) The culture of neural stem cells. *Journal of cellular biochemistry* **106:** 1–6

Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, Hodges AK, Davies DR, David SS, Sampson JR & Cheadle JP (2002) Inherited variants of MYH associated with somatic G:C-->T:A mutations in colorectal tumors. *Nature genetics* **30:** 227–32

Arai T, Kelly VP, Minowa O, Noda T & Nishimura S (2006) The study using wild-type and Ogg1 knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA. *Toxicology* **221:** 179–86

Arvidsson A, Collin T, Kirik D, Kokaia Z & Lindvall O (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature medicine* **8:** 963–70

Audebert M, Radicella JP & Dizdaroglu M (2000) Effect of single mutations in the OGG1 gene found in human tumors on the substrate specificity of the Ogg1 protein. *Nucleic acids research* **28:** 2672–8

Bailly V, Derydt M & Verly WG (1989) Delta-elimination in the repair of AP (apurinic/apyrimidinic) sites in DNA. *The Biochemical journal* **261:** 707–13

Bailly V & Verly WG (1987) Escherichia coli endonuclease III is not an endonuclease but a beta-elimination catalyst. *The Biochemical journal* **242:** 565–72

Bandaru V, Sunkara S, Wallace SS & Bond JP (2002) A novel human DNA glycosylase that removes oxidative DNA damage and is homologous to Escherichia coli endonuclease VIII. *DNA Repair* **1:** 517–529

Bandaru V, Zhao X, Newton MR, Burrows CJ & Wallace SS (2007) Human endonuclease VIII-like (NEIL) proteins in the giant DNA Mimivirus. *DNA Repair* **6:** 1629–1641

Banerjee A, Santos WL & Verdine GL (2006) Structure of a DNA glycosylase searching for lesions. *Science* **311:** 1153–7

Banerjee D, Mandal SM, Das A, Hegde ML, Das S, Bhakat KK, Boldogh I, Sarkar PS, Mitra S & Hazra TK (2011) Preferential repair of oxidized base damage in the transcribed genes of mammalian cells. *The Journal of biological chemistry* **286:** 6006–16

Barnes DE & Lindahl T (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annual review of genetics* **38:** 445–76

Barry KH, Koutros S, Berndt SI, Andreotti G, Hoppin JA, Sandler DP, Burdette LA, Yeager M, Freeman LEB, Lubin JH, Ma X, Zheng T & Alavanja MCR (2011) Genetic variation in base excision repair pathway genes, pesticide exposure, and prostate cancer risk. *Environmental health perspectives* **119:** 1726–32

Le Belle JE, Orozco NM, Paucar AA, Saxe JP, Mottahedeh J, Pyle AD, Wu H & Kornblum HI (2011) Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. *Cell stem cell* **8:** 59–71

Bhakat KK, Hazra TK & Mitra S (2004) Acetylation of the human DNA glycosylase NEIL2 and inhibition of its activity. *Nucleic acids research* **32:** 3033–9

Bhakat KK, Mokkapati SK, Boldogh I, Hazra TK & Mitra S (2006) Acetylation of Human 8-Oxoguanine-DNA Glycosylase by p300 and Its Role in 8-Oxoguanine Repair In Vivo. *Molecular and Cellular Biology* **26:** 1654–1665

Bjorâs M, Luna L, Johnsen B, Hoff E, Haug T, Rognes T & Seeberg E (1997) Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites. *The EMBO journal* **16:** 6314–22

Black HS (1987) Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. *Photochemistry and photobiology* **46:** 213–21

Blainey PC, Luo G, Kou SC, Mangel WF, Verdine GL, Bagchi B & Xie XS (2009) Nonspecifically bound proteins spin while diffusing along DNA. *Nature structural & molecular biology* **16:** 1224–9

Blainey PC, van Oijen AM, Banerjee A, Verdine GL & Xie XS (2006) A base-excision DNA-repair protein finds intrahelical lesion bases by fast sliding in contact with DNA. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 5752–7

Bohr VA, Ottersen OP & Tønjum T (2007) Genome instability and DNA repair in brain, ageing and neurological disease. *Neuroscience* **145:** 1183–6

Brettel K & Byrdin M (2010) Reaction mechanisms of DNA photolyase. *Current opinion in structural biology* **20:** 693–701

Broderick P, Bagratuni T, Vijayakrishnan J, Lubbe S, Chandler I & Houlston RS (2006) Evaluation of NTHL1, NEIL1, NEIL2, MPG, TDG, UNG and SMUG1 genes in familial colorectal cancer predisposition. *BMC cancer* **6:** 243

Brown JA, Pack LR, Sanman LE & Suo Z (2011) Efficiency and fidelity of human DNA polymerases λ and β during gap-filling DNA synthesis. *DNA repair* **10:** 24–33

Brunt KR, Weisel RD & Li R (2012) Stem cells and regenerative medicine — future perspectives. *Canadian Journal of Physiology and Pharmacology* **335:** 327–335

Burgess S, Jaruga P, Dodson ML, Dizdaroglu M & Lloyd RS (2002) Determination of active site residues in Escherichia coli endonuclease VIII. *Journal of bacteriology.* **277:** 2938–2944

Burkovics P, Hajdú I, Szukacsov V, Unk I & Haracska L (2009) Role of PCNA-dependent stimulation of 3'-phosphodiesterase and 3'-5' exonuclease activities of human Ape2 in repair of oxidative DNA damage. *Nucleic acids research* **37:** 4247–55

Burney S, Caulfield JL, Niles JC, Wishnok JS & Tannenbaum SR (1999) The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutation research* **424:** 37–49

Caldecott KW, Aoufouchi S, Johnson P & Shall S (1996) XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular "nick-sensor" in vitro. *Nucleic acids research* **24:** 4387–94

Campalans A, Marsin S, Nakabeppu Y, O'connor TR, Boiteux S & Radicella JP (2005) XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. *DNA repair* **4:** 826–35

Canugovi C, Yoon JS, Feldman NH, Croteau DL, Mattson MP & Bohr VA (2012) Endonuclease VIII-like 1 (NEIL1) promotes short-term spatial memory retention and protects from ischemic stroke-induced brain dysfunction and death in mice. *Proceedings of the National Academy of Sciences of the United States of America* **1:** 1–6

Cardozo-pelaez F, Sanchez-contreras M & Nevin ABC (2012) Ogg1 null mice exhibit age-associated loss of the nigrostriatal pathway and increased sensitivity to MPTP. *Neurochemistry International* **61:** 721–730

Chan MK, Ocampo-Hafalla MT, Vartanian V, Jaruga P, Kirkali G, Koenig KL, Brown S, Lloyd RS, Dizdaroglu M & Teebor GW (2009) Targeted deletion of the genes encoding NTH1 and NEIL1 DNA N-glycosylases reveals the existence of novel carcinogenic oxidative damage to DNA. *DNA repair* **8:** 786–94

Chen DS, Herman T & Demple B (1991) Two distinct human DNA diesterases that hydrolyze 3'-blocking deoxyribose fragments from oxidized DNA. *Nucleic acids research* **19:** 5907–14

Cheng KC, Cahill DS, Kasai H, Nishimura S & Loeb LA (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G----T and A----C substitutions. *The Journal of biological chemistry* **267:** 166–72

Chetsanga CJ & Lindahl T (1979) Release of l-methylguanine residues whose imidazole rings have been opened from damaged DNA by a DNA glycosylase from Escherichia coli. *Nucleic Acids Research* **6:** 3673–3684

Cho YM, Kwon S, Pak YK, Seol HW, Choi YM, Park DJ, Park KS & Lee HK (2006) Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the

spontaneous differentiation of human embryonic stem cells. *Biochemical and biophysical research communications* **348:** 1472–8

Clauson CL, Oestreich KJ, Austin JW & Doetsch PW (2010) Abasic sites and strand breaks in DNA cause transcriptional mutagenesis in Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 3657–62

Coppedè F, Ceravolo R, Migheli F, Fanucchi F, Frosini D, Siciliano G, Bonuccelli U & Migliore L (2010) The hOGG1 Ser326Cys polymorphism is not associated with sporadic Parkinson's disease. *Neuroscience letters* **473:** 248–51

Coppedè F, Mancuso M, Lo Gerfo A, Carlesi C, Piazza S, Rocchi A, Petrozzi L, Nesti C, Micheli D, Bacci A, Migliore L, Murri L & Siciliano G (2007a) Association of the hOGG1 Ser326Cys polymorphism with sporadic amyotrophic lateral sclerosis. *Neuroscience letters* **420:** 163–8

Coppedè F, Mancuso M, Lo Gerfo A, Manca ML, Petrozzi L, Migliore L, Siciliano G & Murri L (2007b) A Ser326Cys polymorphism in the DNA repair gene hOGG1 is not associated with sporadic Alzheimer's disease. *Neuroscience letters* **414:** 282–5

Coppedè F & Migliore L (2009) DNA damage and repair in Alzheimer's disease. *Current Alzheimer Research* **6:** 36–47

Cortázar D, Kunz C, Selfridge J, Lettieri T, Saito Y, MacDougall E, Wirz A, Schuermann D, Jacobs AL, Siegrist F, Steinacher R, Jiricny J, Bird A & Schär P (2011) Embryonic lethal phenotype reveals a function of TDG in maintaining epigenetic stability. *Nature* **470:** 419–23

Crick F (1974) The double helix: a personal view. *Nature* **248:** 766 – 769

Dahlmann HA, Vaidyanathan VG & Sturla SJ (2010) Investigating the Biochemical Impact of DNA Damage with Structure-Based Probes: Abasic Sites, Photodimers, Alkylation Adducts, and Oxidative Lesions. *National Institute of Health* **48:** 9347–9359

Dalhus B, Laerdahl JK, Backe PH & Bjørås M (2009) DNA base repair--recognition and initiation of catalysis. *FEMS microbiology reviews* **33:** 1044–78

Dallosso AR, Dolwani S, Jones N, Jones S, Colley J, Maynard J, Idziaszczyk S, Humphreys V, Arnold J, Donaldson A, Eccles D, Ellis A, Evans DG, Frayling IM, Hes FJ, Houlston RS, Maher ER, Nielsen M, Parry S, Tyler E, *et al* (2008) Inherited predisposition to colorectal adenomas caused by multiple rare alleles of MUTYH but not OGG1, NUDT1, NTH1 or NEIL 1, 2 or 3. *Gut* **57:** 1252–1255

Delaney S, Neeley WL, Delaney JC & Essigmann JM (2007) The substrate specificity of MutY for hyperoxidized guanine lesions in vivo. *Biochemistry* **46:** 1448–55

Dhénaut a, Boiteux S & Radicella JP (2000) Characterization of the hOGG1 promoter and its expression during the cell cycle. *Mutation research* **461:** 109–18

Dianov G & Lindahl T (1994) Reconstitution of the DNA base excision-repair pathway. *Current biology* **4:** 1069–76

Diderich K, Alanazi M & Hoeijmakers JHJ (2011) Premature aging and cancer in nucleotide excision repair-disorders. *DNA repair* **10:** 772–80

Dizdaroglu M, Karahalil B, Sentürker S, Buckley TJ & Roldán-Arjona T (1999) Excision of products of oxidative DNA base damage by human NTH1 protein. *Biochemistry* **38:** 243–6

Dou H, Mitra S & Hazra TK (2003a) Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2. *The Journal of biological chemistry* **278:** 49679–84

Dou H, Mitra S & Hazra TK (2003b) Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2. *The Journal of biological chemistry* **278:** 49679–49684

Dou H, Theriot C a, Das A, Hegde ML, Matsumoto Y, Boldogh I, Hazra TK, Bhakat KK & Mitra S (2008) Interaction of the human DNA glycosylase NEIL1 with proliferating cell nuclear antigen. The potential for replication-associated repair of oxidized bases in mammalian genomes. *The Journal of biological chemistry* **283:** 3130–40

Doublie S, Bandaru V, Bond JP & Wallace SS (2004) The crystal structure of human endonuclease VIII-like 1 (NEIL1) reveals a zincless finger motif required for glycosylase activity. *Proceedings of the National Academy of Sciences of the United States of America* **101:** 10284–10289

Drabløs F, Feyzi E, Aas PA, Vaagbø CB, Kavli B, Bratlie MS, Peña-Diaz J, Otterlei M, Slupphaug G & Krokan HE (2004) Alkylation damage in DNA and RNA--repair mechanisms and medical significance. *DNA repair* **3:** 1389–407

Duarte V, Muller JG & Burrows CJ (1999) Insertion of dGMP and dAMP during in vitro DNA synthesis opposite an oxidized form of 7,8-dihydro-8-oxoguanine. *Nucleic Acids Research* **27:** 496–502

Dunn AR, Kad NM, Nelson SR, Warshaw DM & Wallace SS (2011) Single Qdot-labeled glycosylase molecules use a wedge amino acid to probe for lesions while scanning along DNA. *Nucleic acids research* **39:** 7487–98

Eker APM, Quayle C, Chaves I & van der Horst GTJ (2009) DNA repair in mammalian cells: Direct DNA damage reversal: elegant solutions for nasty problems. *Cellular and molecular life sciences* **66:** 968–80

Endres M, Biniszkiewicz D, Sobol RW, Harms C, Ahmadi M, Lipski A, Katchanov J, Mergenthaler P, Dirnagl U, Wilson SH, Meisel A & Jaenisch R (2004) Increased postischemic brain injury in mice deficient in uracil-DNA glycosylase. *The Journal of Clinical Investigation* **113:** 1711–1721

Espeseth AS, Fishel R, Hazuda D, Huang Q, Xu M, Yoder K & Zhou H (2011) siRNA screening of a targeted library of DNA repair factors in HIV infection reveals a role for base excision repair in HIV integration. *PloS one* **6:** e17612

Falnes PØ, Klungland A & Alseth I (2007) Repair of methyl lesions in DNA and RNA by oxidative demethylation. *Neuroscience* **145:** 1222–32

Fan J & Wilson DM (2005) Protein-protein interactions and posttranslational modifications in mammalian base excision repair. *Free radical biology & medicine* **38:** 1121–38

Forsbring M, Vik ES, Dalhus B, Karlsen TH, Bergquist A, Schrumpf E, Bjørås M, Boberg KM & Alseth I (2009) Catalytically impaired hMYH and NEIL1 mutant proteins identified in patients with primary sclerosing cholangitis and cholangiocarcinoma. *Carcinogenesis* **30:** 1147–54

Fortini P & Dogliotti E (2007) Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. *DNA repair* **6:** 398–409

Freeman MR (2010) Specification and morphogenesis of astrocytes. *Science* **330:** 774–8

Friedman JI & Stivers JT (2010) Detection of damaged DNA bases by DNA glycosylase enzymes. *Biochemistry* **49:** 4957–67

Fromme JC, Banerjee A & Verdine GL (2004) DNA glycosylase recognition and catalysis. *Current opinion in structural biology* **14:** 43–9

Gao Y, Katyal S, Lee Y, Zhao J, Rehg JE, Russell HR & McKinnon PJ (2011) DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair. *Nature* **471:** 240–4

Guikema JEJ, Gerstein RM, Linehan EK, Cloherty EK, Evan-Browning E, Tsuchimoto D, Nakabeppu Y & Schrader CE (2011) Apurinic/apyrimidinic endonuclease 2 is necessary for normal B cell development and recovery of lymphoid progenitors after chemotherapeutic challenge. *Journal of immunology* **186:** 1943–50

Hadi MZ & Wilson DM (2000) Second human protein with homology to the Escherichia coli abasic endonuclease exonuclease III. *Environmental and molecular mutagenesis* **36:** 312–24

Hailer MK, Slade PG, Martin BD, Rosenquist TA & Sugden KD (2005a) Recognition of the oxidized lesions spiroiminodihydantoin and guanidinohydantoin in DNA by the mammalian base excision repair glycosylases NEIL1 and NEIL2. *DNA repair* **4:** 41–50

Hailer MK, Slade PG, Martin BD & Sugden KD (2005b) Nei Deficient Escherichia coli Are Sensitive to Chromate and Accumulate the Oxidized Guanine Lesion Spiroiminodihydantoin. *Chemical research in toxicology* **18:** 1378–1383

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? *Journal of neurochemistry* **97:** 1634–58

Harman D (1981) The aging process. *Proceedings of the National Academy of Sciences of the United States of America* **78:** 7124–7128

Hartlerode AJ & Scully R (2009) Mechanisms of double-strand break repair in somatic mammalian cells. *The Biochemical journal* **423:** 157–68

Haynes C, Oldfield CJ, Ji F, Klitgord N, Cusick ME, Radivojac P, Uversky VN, Vidal M & Iakoucheva LM (2006) Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes. *PLoS computational biology* **2:** 890–902

Hazra TK, Izumi T, Boldogh I, Imhoff B, Kow YW, Jaruga P, Dizdaroglu M & Mitra S (2002a) Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. *Proceedings of the National Academy of Sciences of the United States of America* **99:** 3523–3528

Hazra TK, Kow YW, Hatahet Z, Imhoff B, Boldogh I, Mokkapati SK, Mitra S & Izumi T (2002b) Identification and characterization of a novel human DNA glycosylase for repair of cytosine-derived lesions. *Journal of Biological Chemistry.* **277:** 30417–30420

Hegde ML, Hazra TK & Mitra S (2008a) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. *Cell research* **18:** 27–47

Hegde ML, Hazra TK & Mitra S (2010) Functions of disordered regions in mammalian early base excision repair proteins. *Cellular and molecular life sciences* **67:** 3573–87

Hegde ML, Mantha AK, Hazra TK, Bhakat KK, Mitra S & Szczesny B (2012) Oxidative genome damage and its repair: Implications in aging and neurodegenerative diseases. *Mechanisms of ageing and development* **133:** 157–168

Hegde ML, Theriot CA, Das A, Hegde PM, Guo Z, Gary RK, Hazra TK, Shen B & Mitra S (2008b) Physical and functional interaction between human oxidized base-specific DNA glycosylase NEIL1 and flap endonuclease 1. *The Journal of biological chemistry* **283:** 27028–37

Helbock HJ, Beckman KB, Shigenaga MK, Walter PB, Woodall a a, Yeo HC & Ames BN (1998) DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proceedings of the National Academy of Sciences of the United States of America* **95:** 288–93

Henderson PT, Delaney JC, Gu F, Tannenbaum SR & Essigmann JM (2002) Oxidation of 7,8-dihydro-8-oxoguanine affords lesions that are potent sources of replication errors in vivo. *Biochemistry* **41:** 914–21

Henderson PT, Delaney JC, Muller JG, Neeley WL, Tannenbaum SR, Burrows CJ & Essigmann JM (2003) The hydantoin lesions formed from oxidation of 7,8-dihydro-8-oxoguanine are potent sources of replication errors in vivo. *Biochemistry* **42:** 9257–62

Hendrich B, Hardeland U, Ng HH, Jiricny J & Bird A (1999) The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. *Nature* **401:** 301–4

Hildrestrand GA, Neurauter CG, Diep DB, Castellanos CG, Krauss S, Bjoras M & Luna L (2009) Expression patterns of Neil3 during embryonic brain development and neoplasia. *BMC Neuroscience* **10:** 45

Hoeijmakers JHJ (2001) Genome maintenance mechanisms for preventing cancer. *Nature* **411:** 366–374

Hoeijmakers JHJ (2009) DNA damage, aging, and cancer. *The New England journal of medicine* **361:** 1475–85

Ide Y, Tsuchimoto D, Tominaga Y, Nakashima M, Watanabe T, Sakumi K, Ohno M & Nakabeppu Y (2004) Growth retardation and dyslymphopoiesis accompanied by G2/M arrest in APEX2-null mice. *Blood* **104:** 4097–103

Imai K, Sarker AH, Akiyama K, Ikeda S, Yao M, Tsutsui K, Shohmori T & Seki S (1998) Genomic structure and sequence of a human homologue (NTHL1/NTH1) of Escherichia coli endonuclease III with those of the adjacent parts of TSC2 and SLC9A3R2 genes. *Gene* **222:** 287–95

Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y & Suda T (2006) Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nature medicine* **12:** 446–51

Iwakuma T, Sakumi K, Nakatsuru Y, Kawate H, Igarashi H, Shiraishi A, Tsuzuki T, Ishikawa T & Sekiguchi M (1997) High incidence of nitrosamine-induced tumorigenesis in mice lacking DNA repair methyltransferase. *Carcinogenesis* **18:** 1631–5

Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY & Semenza GL (1998) Cellular and developmental control of O 2 homeostasis by hypoxia-inducible factor 1. *Genes and development* **12:** 149–162

Jacobs AL & Schär P (2012) DNA glycosylases: in DNA repair and beyond. *Chromosoma* **121:** 1–20

Jasperson KW, Tuohy TM, Neklason DW & Burt RW (2010) Hereditary and familial colon cancer. *Gastroenterology* **138:** 2044–58

Jensen JB & Parmar M (2006) Strengths and limitations of the neurosphere culture system. *Molecular neurobiology* **34:** 153–61

Jeppesen DK, Bohr VA & Stevnsner T (2011) DNA repair deficiency in neurodegeneration. *Progress in neurobiology* **94:** 166–200

Jiricny J (2006) The multifaceted mismatch-repair system. *Nature reviews. Molecular cell biology* **7:** 335–46

Jones S, Emmerson P, Maynard J, Best JM, Jordan S, Williams GT, Sampson JR & Cheadle JP (2002) Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C-->T:A mutations. *Human molecular genetics* **11:** 2961–7

Kanavy HE & Gerstenblith MR (2011) Ultraviolet radiation and melanoma. *Seminars in cutaneous medicine and surgery* **30:** 222–8

Karahalil B, de Souza-Pinto NC, Parsons JL, Elder RH & Bohr VA (2003) Compromised incision of oxidized pyrimidines in liver mitochondria of mice deficient in NTH1 and OGG1 glycosylases. *The Journal of biological chemistry* **278:** 33701–7

Karimi-Busheri F, Daly G, Robins P, Canas B, Pappin DJC, Sgouros J, Miller GG, Fakhrai H, Davis EM, Beau MML & Weinfeld M (1999) Molecular Characterization of a Human DNA Kinase. *The Journal of biological chemistry* **274:** 24187–24194

Kasai H, Nishimura S & Division B (1984) Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic acid research* **12:** 2137–2145

Kasparek TR & Humphrey TC (2011) DNA double-strand break repair pathways, chromosomal rearrangements and cancer. *Seminars in cell & developmental biology* **22:** 886–97

Kauffmann A, Rosselli F, Lazar V, Winnepenninckx V, Mansuet-Lupo A, Dessen P, van den Oord JJ, Spatz A & Sarasin A (2008) High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene* **27:** 565–73

Klaunig JE, Wang Z, Pu X & Zhou S (2011) Oxidative stress and oxidative damage in chemical carcinogenesis. *Toxicology and applied pharmacology* **254:** 86–99

Klein JC, Bleeker MJ, Saris CP, Roelen HC, Brugghe HF, van den Elst H, van der Marel GA, van Boom JH, Westra JG & Kriek E (1992) Repair and replication of plasmids with site-specific 8-oxodG and 8-AAFdG residues in normal and repair-deficient human cells. *Nucleic acids research* **20:** 4437–43

Klungland A & Lindahl T (1997) Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *The EMBO journal* **16:** 3341–8

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T & Barnes DE (1999) Accumulation of premutagenic DNA lesions in mice defective in

removal of oxidative base damage. *Proceedings of the National Academy of Sciences of the United States of America* **96:** 13300–13305

Kow YW & Wallace SS (1987) Mechanism of action of Escherichia coli endonuclease III. *Biochemistry* **26:** 8200–8206

Krishnamurthy N, Zhao X, Burrows CJ & David SS (2008) Superior Removal of Hydantoin Lesions Relative to Other Oxidized Bases by the Human DNA Glycosylase hNEIL1. *Biochemistry* **47:** 7137–7146

Krokan HE, Standal R & Slupphaug G (1997) DNA glycosylases in the base excision repair of DNA. *Biochemical Journal* **16:** 1–16

Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE & Lindahl T (1996) Reconstitution excision-repair purified proteins: polymerase protein. *The EMBO journal* **15:** 6662–6670

Lan J, Li W, Zhang F, Sun FY, Nagayama T, O'Horo C & Chen J (2003) Inducible repair of oxidative DNA lesions in the rat brain after transient focal ischemia and reperfusion. *Journal of cerebral blood flow and metabolism* **23:** 1324–39

Lau a Y, Schärer OD, Samson L, Verdine GL & Ellenberger T (1998) Crystal structure of a human alkylbase-DNA repair enzyme complexed to DNA: mechanisms for nucleotide flipping and base excision. *Cell* **95:** 249–58

Lawson LJ, Perry VH & Gordon S (1992) Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* **48:** 405–15

Li H, Hao X, Zhang W, Wei Q & Chen K (2008) The hOGG1 Ser326Cys polymorphism and lung cancer risk: a meta-analysis. *Cancer epidemiology, biomarkers & prevention* **17:** 1739–45

Li P, Hu X, Gan Y, Gao Y, Liang W & Chen J (2011) Mechanistic insight into DNA damage and repair in ischemic stroke: exploiting the base excision repair pathway as a model of neuroprotection. *Antioxidants & redox signaling* **14:** 1905–18

Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature* **362:** 13300–13305

Lindahl T & Nyberg B (1972) Rate of depurination of native deoxiribonucleic acid. *Biochemistry* **11:** 3610–3618

Liu D, Croteau DL, Souza-Pinto N, Pitta M, Tian J, Wu C, Jiang H, Mustafa K, Keijzers G, Bohr VA & Mattson MP (2011) Evidence that OGG1 glycosylase protects neurons against oxidative DNA damage and cell death under ischemic conditions. *Journal of cerebral blood flow and metabolism* **31:** 680–92

Liu M, Bandaru V, Bond JP, Jaruga P, Zhao X, Christov PP, Burrows CJ, Rizzo CJ, Dizdaroglu M & Wallace SS (2010) The mouse ortholog of NEIL3 is a functional DNA glycosylase in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 4925–4930

Liu M, Bandaru V, Holmes A, Averill AM, Cannan W & Wallace SS (2012) Expression and purification of active mouse and human NEIL3 proteins. *Protein expression and purification* **84:** 130–139

Liu X & Roy R (2002) Truncation of Amino-terminal Tail Stimulates Activity of Human Endonuclease III (hNTH1). *Journal of Molecular Biology* **321:** 265–276

Lodish H, Berk A, Matsudeira P, Kaiser C, Kreiger M & Scott M (2004) Molecular cell biology

van Loon B, Markkanen E & Hübscher U (2010) Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. *DNA repair* **9:** 604–16

Ludwig D, MacInnes M, Takiguchi Y, Purtymun P, Henrie M, Flannery M, Meneses J, Pedersen R & Chen D (1998) A murine AP-endonuclease gene-targeted deficiency with post-implantation embryonic progression and ionizing radiation sensitivity. *Mutatation research* **1:** 17–29

Luna L, Bjørås M, Hoff E, Rognes T & Seeberg E (2000) Cell-cycle regulation, intracellular sorting and induced overexpression of the human NTH1 DNA glycosylase involved in removal of formamidopyrimidine residues from DNA. *Mutation research* **460:** 95–104

Magistretti PJ & Pellerin L (1996) Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a prominent role of astrocytes. *Cerebral cortex* **6:** 50–61

Mangerich A, Knutson CG, Parry NM, Muthupalani S, Ye W & Prestwich E (2012) Infection-induced colitis in mice causes dynamic and tissue-specific changes in stress response and DNA damage leading to colon cancer. *Proceedings of the National Academy of Sciences of the United States of America* **109:**

Mao G, Pan X, Zhu B-B, Zhang Y, Yuan F, Huang J, Lovell MA, Lee MP, Markesbery WR, Li GM & Gu L (2007) Identification and characterization of OGG1 mutations in patients with Alzheimer's disease. *Nucleic acids research* **35:** 2759–66

Marenstein DR, Ocampo MT, Chan MK, Altamirano A, Basu AK, Boorstein RJ, Cunningham RP & Teebor GW (2001) Stimulation of human endonuclease III by Y box-binding protein 1 (DNA-binding protein B). Interaction between a base excision repair enzyme and a transcription factor. *The Journal of biological chemistry* **276:** 21242–9

Martino G, Pluchino S, Bonfanti L & Schwartz M (2011) Brain regeneration in physiology and pathology: the immune signature driving therapeutic plasticity of neural stem cells. *Physiological reviews* **91:** 1281–304

Matsumoto Y & Kim K (1995) Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. *Science* **269:** 699–702

Matsumoto Y, Kim K, Hurwitz J, Gary R, Levin DS, Tomkinson AE & Park MS (1999) Reconstitution of proliferating cell nuclear antigen-dependent repair of apurinic/apyrimidinic sites with purified human proteins. *The Journal of biological chemistry* **274:** 33703–8

Mazumdar J, O'Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS & Simon MC (2010) O2 regulates stem cells through Wnt/β-catenin signalling. *Nature cell biology* **12:** 1007–13

Melamede RJ, Hatahet Z, Kow YW, Ide H & Wallace SS (1994) Isolation and characterization of endonuclease VIII from Escherichia coli. *Biochemistry* **33:** 1255–64

Minowa O, Arai T, Hirano M, Monden Y, Nakai S, Fukuda M, Itoh M, Takano H, Hippou Y, Aburatani H, Masumura K, Nohmi T, Nishimura S & Noda T (2000) Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 4156–4161

Morgan WF & Sowa MB (2005) Effects of ionizing radiation in nonirradiated cells. *Proceedings of the National Academy of Sciences of the United States of America* **102:** 14127–8

Morland I, Luna L, Gustad E, Seeberg E & Bjørås M (2005) Product inhibition and magnesium modulate the dual reaction mode of hOgg1. *DNA repair* **4:** 381–7

Morland I, Rolseth V, Luna L, Rognes T, Bjoras M & Seeberg E (2002) Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. *Nucleic Acids Research* **30:** 4926–4936

Mullen GP & Wilson SH (1997) New Concepts in Biochemistry DNA Polymerase in Abasic Site Repair : A Structurally Conserved Helix-Hairpin-Helix Motif in Lesion Detection by Base Excision Repair. *Biochemistry* **36:** 4713–4725

Muller C, Paupert J, Monferran S & Salles B (2005) The Double Life of the Ku Protein. *Cell cycle* **4:** 438–441

Munk BH, Burrows CJ & Schlegel HB (2008) An exploration of mechanisms for the transformation of 8-oxoguanine to guanidinohydantoin and spiroiminodihydantoin by density functional theory. *Journal of the American Chemical Society* **130:** 5245–56

Nave KA (2010) Myelination and support of axonal integrity by glia. *Nature* **468:** 244–52

Neurauter CG, Luna L & Bjørås M (2012) Release from quiescence stimulates the expression of human NEIL3 under the control of the Ras dependent ERK-MAP kinase pathway. *DNA repair* **11:** 401–9

Niedernhofer LJ, Bohr VA, Sander M & Kraemer KH (2011) Xeroderma pigmentosum and other diseases of human premature aging and DNA repair: molecules to patients. *Mechanisms of ageing and development* **132:** 340–7

Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, Odijk H, Oostendorp R, Ahmad A, van Leeuwen W, Theil AF, Vermeulen W, van der Horst GTJ, Meinecke P, Kleijer WJ, Vijg J, Jaspers NGJ & Hoeijmakers JHJ (2006) A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. *Nature* **444:** 1038–43

Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K & Nakabeppu Y (1999) Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Molecular biology of the cell* **10:** 1637–52

Nitti M, Furfaro AL, Cevasco C, Traverso N, Marinari UM, Pronzato MA & Domenicotti C (2010) PKC delta and NADPH oxidase in retinoic acid-induced neuroblastoma cell differentiation. *Cellular signalling* **22:** 828–35

Nouspikel T (2007) DNA repair in differentiated cells: some new answers to old questions. *Neuroscience* **145:** 1213–21

Nouspikel T & Hanawalt PC (2000) Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. *Molecular and cellular biology* **20:** 1562–70

Nouspikel T & Hanawalt PC (2002) DNA repair in terminally differentiated cells. *DNA repair* **1:** 59–75

Odell ID, Newick K, Heintz NH, Wallace SS & Pederson DS (2010) Non-specific DNA binding interferes with the efficient excision of oxidative lesions from chromatin by the human DNA glycosylase, NEIL1. *DNA repair* **9:** 134–43

Odell ID, Wallace SS & Pederson DS (2012) Rules of engagement for base excision repair in chromatin. *Journal of cellular physiology***:** 1–33

Olinski R, Gackowski D, Foksinski M, Rozalski R, Roszkowski K & Jaruga P (2002) Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. *Free Radical Biology & Medicine* **33:** 192–200

Pascucci B, Stucki M, Jo O, Dogliotti E & Hu U (1999) Long Patch Base Excision Repair with Purified Human Proteins. **274:** 33696–33702

Pawelczak KS, Bennett SM & Turchi JJ (2011) Coordination of DNA-PK activation and nuclease processing of DNA termini in NHEJ. *Antioxidants & redox signaling* **14:** 2531–43

Pearl LH (2000) Structure and function in the uracil-DNA glycosylase superfamily. *Mutation research* **460:** 165–81

Porecha RH & Stivers JT (2008) Uracil DNA glycosylase uses DNA hopping and short-range sliding to trap extrahelical uracils. *Proceedings of the National Academy of Sciences of the United States of America* **105:** 10791–6

Prakash A, Doublié S & Wallace SS (2012) The Fpg/Nei family of DNA glycosylases: substrates, structures, and search for damage. *Progress in molecular biology and translational science* **110:** 71–91

Prasad R, Beard WA, Strauss PR & Wilson SH (1998) Human DNA Polymerase $^{N}_{L}$ Deoxyribose Phosphate Lyase. **273:** 15263–15270

Proctor RN (2012) The history of the discovery of the cigarette-lung cancer link: evidentiary traditions, corporate denial, global toll. *Tobacco control* **21:** 87–91

Puebla-Osorio N, Lacey DB, Alt FW & Zhu C (2006) Early Embryonic Lethality Due to Targeted Inactivation of DNA Ligase III †. **26:** 3935–3941

Rasouli-Nia A, Karimi-Busheri F & Weinfeld M (2004) Stable down-regulation of human polynucleotide kinase enhances spontaneous mutation frequency and sensitizes cells to genotoxic agents. *Proceedings of the National Academy of Sciences of the United States of America* **101:** 6905–10

Redrejo-Rodríguez M, Saint-Pierre C, Couve S, Mazouzi A, Ishchenko AA, Gasparutto D & Saparbaev M (2011) New insights in the removal of the hydantoins, oxidation product of pyrimidines, via the base excision and nucleotide incision repair pathways. *PloS one* **6:** e21039

Regnell CE, Hildrestrand GA, Sejersted Y, Medin T, Moldestad O, Rolseth V, Krokeide SZ, Suganthan R, Luna L, Bjoras M & Bergersen LH (2012) Hippocampal Adult Neurogenesis Is Maintained by Neil3-Dependent Repair of Oxidative DNA Lesions in Neural Progenitor Cells. *Cell* **In Press:** 1–8

Reis A & Hermanson O (2012) The DNA glycosylases OGG1 and NEIL3 influence differentiation potential , proliferation , and senescence-associated signs in neural stem cells. *Biochemical and Biophysical Research Communications***:** 1–6

Reynolds B & Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255:** 1707–1710

Riley P (1994) Free radicals in biology: oxidative stress and the effects of ionizing radiation. *International Journal of Radiation Biology* **65:** 27–33

Roberts RJ & Cheng X (1998) Base flipping. *Annual review of biochemistry* **67:** 181–98

Robertson AB, Klungland A, Rognes T & Leiros I (2009) DNA repair in mammalian cells: Base excision repair: the long and short of it. *Cellular and molecular life sciences* **66:** 981–93

Rocha CRR, Lerner LK, Okamoto OK, Marchetto MC & Menck CFM (2012) The role of DNA repair in the pluripotency and differentiation of human stem cells. *Mutation research***:** 1–11

Rolseth V, Runden-Pran E, Luna L, McMurray C, Bjoras M & Ottersen OP (2008) Widespread distribution of DNA glycosylases removing oxidative DNA lesions in human and rodent brains. *DNA Repair* **7:** 1578–1588

Rosenquist TA, Zaika E, Fernandes AS, Zharkov DO, Miller H & Grollman AP (2003) The novel DNA glycosylase, NEIL1, protects mammalian cells from radiation-mediated cell death. *DNA Repair* **2:** 581–591

Russo MT, De Luca G, Degan P & Bignami M (2007) Different DNA repair strategies to combat the threat from 8-oxoguanine. *Mutation research* **614:** 69–76

Russo MT, De Luca G, Degan P, Parlanti E, Dogliotti E, Barnes DE, Lindahl T, Yang H, Miller JH & Bignami M (2004) Accumulation of the oxidative base lesion 8-hydroxyguanine in DNA of tumor-prone mice defective in both the Myh and Ogg1 DNA glycosylases. *Cancer research* **64:** 4411–4

Rydberg B & Lindahl T (1982) Nonenzymatic methylation of DNA by the intracellular methyl group donor S-adenosyl-L-methionine is a potentially mutagenic reaction. *The EMBO journal* **1:** 211–216

Sabouri Z, Okazaki I-M, Shinkura R, Begum N, Nagaoka H, Tsuchimoto D, Nakabeppu Y & Honjo T (2009) Apex2 is required for efficient somatic hypermutation but not for class switch recombination of immunoglobulin genes. *International immunology* **21:** 947–55

Saeed SA, Shad KF, Saleem T, Javed F & Khan MU (2007) Some new prospects in the understanding of the molecular basis of the pathogenesis of stroke. *Experimental brain research.* **182:** 1–10

Sakaguchi K, Ishibashi T, Uchiyama Y & Iwabata K (2009) The multi-replication protein A (RPA) system--a new perspective. *The FEBS journal* **276:** 943–63

Salganik RI (2001) The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population. *Journal of the American College of Nutrition* **20:** 464S–472S; discussion 473S–475S

Sampath H, Batra AK, Vartanian V, Carmical JR, Prusak D, King IB, Lowell B, Earley LF, Wood TG, Marks DL, McCullough AK & R Stephen L (2011) Variable penetrance of metabolic phenotypes and development of high-fat diet-induced adiposity in NEIL1-deficient mice. *American journal of physiology. Endocrinology and metabolism* **300:** E724–34

Sampath H, McCullough AK & Lloyd RS (2012) Regulation of DNA glycosylases and their role in limiting disease. *Free radical research* **46:** 460–78

Saparbaev M, Sidorkina OM, Jurado J, Privezentzev CV, Greenberg MM & Laval J (2002) Repair of oxidized purines and damaged pyrimidines by E. coli Fpg protein: different roles of proline 2 and lysine 57 residues. *Environmental and molecular mutagenesis* **39:** 10–17

Sauer H, Rahimi G, Hescheler J & Wartenberg M (2000) Role of reactive oxygen species and phosphatidylinositol 3-kinase in cardiomyocyte differentiation of embryonic stem cells. *FEBS letters* **476:** 218–23

Schrader M & Fahimi HD (2006) Peroxisomes and oxidative stress. *Biochimica et biophysica acta* **1763:** 1755–66

Seyde W & Longnecker D (1986) Cerebral oxygen tension in rats during deliberate hypotension with sodium nitroprusside, 2-chloroadenosine, or deep isoflurane anesthesia. *Anesthesiology* **64:** 480–485

Shibutani S, Takeshita T & Grollman AP (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349:** 431–434

Sick TJ, Lutz PL, Lamanna JC, Rosenthal M & Lamanna C (1982) Comparative brain oxygenation and mitochondrial redox activity in turtles and rats. *Journal of applied physiology* **53:** 1354–1359

Sidorenko VS, Grollman AP, Jaruga P, Dizdaroglu M & Zharkov DO (2009) Substrate specificity and excision kinetics of natural polymorphic variants and phosphomimetic mutants of human 8-oxoguanine-DNA glycosylase. *The FEBS journal* **276:** 5149–62

Simon MC & Keith B (2008) The role of oxygen availability in embryonic development and stem cell function. *Nature reviews. Molecular cell biology* **9:** 285–96

Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis AK, Van Houten B, Shuman S, McKinnon PJ & Jasin M (2011) Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. *Nature* **471:** 245–8

Slupphaug G, Mol DC, Kavli B, Arvai AS, Krokan HE & Taine JA (1996) A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA. *Nature* **384:** 87–92

Sobol RW, Horton JK, Kühn R, Gu H, Singhel RK, Prasad R, Rajewsky K & Wilson SH (1996) Requirement of mammalian DNA polymerase-beta in base-excision repair. *Nature* **379:** 183–186

Sofroniew MV & Vinters HV (2010) Astrocytes: biology and pathology. *Acta neuropathologica* **119:** 7–35

Soppa J (2010) Protein acetylation in archaea, bacteria, and eukaryotes. *Archaea* **1:** 1–9

Spitzer NC (2006) Electrical activity in early neuronal development. *Nature* **444:** 707–12

Strauss PR, Beard WA, Patterson TA & Wilson SH (1997) Substrate binding by human apurinic/apyrimidinic endonuclease indicates a Briggs-Haldane mechanism. *The Journal of biological chemistry* **272:** 1302–7

Sugahara M, Mikawa T, Kumasaka T, Yamamoto M, Kato R, Fukuyama K, Inoue Y & Kuramitsu S (2000) Crystal structure of a repair enzyme of oxidatively damaged DNA, MutM (Fpg), from an extreme thermophile, Thermus thermophilus HB8. *The EMBO journal* **19:** 3857–69

Sugo N, Aratani Y, Nagashima Y, Kubota Y & Koyama H (2000) Neonatal lethality with abnormal neurogenesis in mice deficient in DNA polymerase beta. *The EMBO journal* **19:** 1397–404

Takao M, Kanno S, Shiromoto T, Hasegawa R, Ide H, Ikeda S, Sarker AH, Seki S, Xing JZ, Le XC, Weinfeld M, Kobayashi K, Miyazaki J, Muijtjens M, Hoeijmakers JHJ, van der Horst G, Yasui A & Sarker AH (2002a) Novel nuclear and mitochondrial glycosylases revealed by disruption of the mouse Nth1 gene encoding an endonuclease III homolog for repair of thymine glycols. *The EMBO journal* **21:** 3486–93

Takao M, Kanno S-I, Kobayashi K, Zhang Q-M, Yonei S, van der Horst GTJ & Yasui A (2002b) A back-up glycosylase in Nth1 knock-out mice is a functional Nei (endonuclease VIII) homologue. *The Journal of biological chemistry* **277:** 42205–13

Takao M, Oohata Y, Kitadokoro K, Kobayashi K, Iwai S, Yasui A, Yonei S & Zhang QM (2009) Human Nei-like protein NEIL3 has AP lyase activity specific for single-stranded DNA and confers oxidative stress resistance in Escherichia coli mutant. *Genes Cells* **14:** 261–270

Taverna P & Sedgwick B (1996) Generation of an endogenous DNA-methylating agent by nitrosation in Escherichia coli. *Journal of bacteriology* **178:** 5105–11

Tchous J, Bodepudi V, Shibutani S, Millers J, Grollmanll AP & Johnson F (1994) Substrate Specificity of Fpg Protein. *The journal of biological chemistry* **269:** 15318–15324

Tebbs RS, Flannery ML, Meneses JJ, Hartmann a, Tucker JD, Thompson LH, Cleaver JE & Pedersen R a (1999) Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. *Developmental biology* **208:** 513–29

Theriot CA, Hegde ML, Hazra TK & Mitra S (2010) RPA physically interacts with the human DNA glycosylase NEIL1 to regulate excision of oxidative DNA base damage in primer-template structures. *DNA repair* **9:** 643–52

Thompson LH & Schild D (2002) Recombinational DNA repair and human disease. *Mutation research* **509:** 49–78

Tokmakov AA, Kurotani A, Takagi T, Toyama M, Shirouzu M, Fukami Y & Yokoyama S (2012) Multiple post-translational modifications bear upon heterologous protein synthesis. *The Journal of biological chemistry* **In Press:**

Tomkinson AE & Mackey ZB (1998) Structure and function of mammalian DNA ligases. *Mutation research Res* **407:** 1–9

Torisu K, Tsuchimoto D, Ohnishi Y & Nakabeppu Y (2005) Hematopoietic tissue-specific expression of mouse Neil3 for endonuclease VIII-like protein. *J.Biochem.* **138:** 763–772

Tremblay M-È, Stevens B, Sierra A, Wake H, Bessis A & Nimmerjahn A (2011) The role of microglia in the healthy brain. *The Journal of neuroscience* **31:** 16064–9

Tsatmali M, Walcott EC, Makarenkova H & Crossin KL (2006) Reactive oxygen species modulate the differentiation of neurons in clonal cortical cultures. *Molecular and cellular neurosciences* **33:** 345–57

Tóth-Petróczy A, Simon I, Fuxreiter M & Levy Y (2009) Disordered tails of homeodomains facilitate DNA recognition by providing a trade-off between folding and specific binding. *Journal of the American Chemical Society* **131:** 15084–5

Uppu R, Cueto R, Squadrito G, Salgo M & Pryor W (1996) Competitive reactions of peroxynitrite with 2'-deoxyguanosine and 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxodG): relevance to the formation of 8-oxodG in DNA exposed to peroxynitrite. *Free radical biology & medicine* **21:** 3–7

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M & Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology* **39:** 44–84

Vartanian V, Lowell B, Minko IG, Wood TG, Ceci JD, George S, Ballinger SW, Corless CL, McCullough AK & Lloyd RS (2006) The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 1864–9

Varum S, Momcilović O, Castro C, Ben-Yehudah A, Ramalho-Santos J & Navara CS (2009) Enhancement of human embryonic stem cell pluripotency through inhibition of the mitochondrial respiratory chain. *Stem cell research* **3:** 142–56

Vasen HF, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, Nagengast FM, Meijers-Heijboer EH, Bertario L, Varesco L, Bisgaard ML, Mohr J, Fodde R & Khan

PM (1996) Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* **110:** 1020–7

Venitt S (1996) Mechanisms of spontaneous human cancers. *Environmental health perspectives* **104:** 633–7

Verbeek B, Southgate TD, Gilham DE & Margison GP (2008) O6-Methylguanine-DNA methyltransferase inactivation and chemotherapy. *British medical bulletin* **85:** 17–33

Vermeulen W (2011) Dynamics of mammalian NER proteins. *DNA repair* **10:** 760–71

Vieira HLA, Alves PM & Vercelli A (2011) Modulation of neuronal stem cell differentiation by hypoxia and reactive oxygen species. *Progress in neurobiology* **93:** 444–55

Vik ES, Alseth I, Forsbring M, Helle IH, Morland I, Luna L, Bjørås M & Dalhus B (2012) Biochemical mapping of human NEIL1 DNA glycosylase and AP lyase activities. *DNA repair* **11:** 766–73

Visnes T, Doseth B, Pettersen HS, Hagen L, Sousa MML, Akbari M, Otterlei M, Kavli B, Slupphaug G & Krokan HE (2009) Uracil in DNA and its processing by different DNA glycosylases. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **364:** 563–8

Vogt S, Jones N, Christian D, Engel C, Nielsen M, Kaufmann A, Steinke V, Vasen HF, Propping P, Sampson JR, Hes FJ & Aretz S (2009) Expanded extracolonic tumor spectrum in MUTYH-associated polyposis. *Gastroenterology* **137:** 1976–85.e1–10

Vuzman D, Azia A & Levy Y (2010) Searching DNA via a "Monkey Bar" mechanism: the significance of disordered tails. *Journal of molecular biology* **396:** 674–84

Wallace SS, Bandaru V, Kathe SD & Bond JP (2003) The enigma of endonuclease VIII. *DNA Repair* **2:** 441–453

Wallace SS, Murphy DL & Sweasy JB (2012) Base excision repair and cancer. *Cancer letters* **In Press:**

Wanders RJA & Waterham HR (2006) Biochemistry of mammalian peroxisomes revisited. *Annual review of biochemistry* **75:** 295–332

Wang W, Esbensen Y, Kunke D, Suganthan R, Rachek L, Bjørås M & Eide L (2011) Mitochondrial DNA damage level determines neural stem cell differentiation fate. *The Journal of neuroscience* **31:** 9746–51

Wang W, Osenbroch P, Skinnes R, Esbensen Y, Bjørås M & Eide L (2010) Mitochondrial DNA integrity is essential for mitochondrial maturation during differentiation of neural stem cells. *Stem cells* **28:** 2195–204

Wang Z, Wang F, Tang T & Guo C (2012) The role of PARP1 in the DNA damage response and its application in tumor therapy. *Frontiers of medicine* **6:** 156–64

Ward J (1988) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. *Progress in Nucleic Acid Research and Molecular Biology* **35:** 95–125

Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF & Jones DT (2004) Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *Journal of molecular biology* **337:** 635–45

Wardman P & Chandeias LP (1996) Fenton chemistry: an introduction. *Radiation Research* **145:** 523–31

Watson J & Crick F (1953) Molecular structure of nucleic acids. *Nature* **171:** 737–738

Weiss JM, Goode EL, Ladiges WC & Ulrich CM (2005) Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. *Molecular carcinogenesis* **42:** 127–41

Wiederhold L, Leppard JB, Kedar P, Karimi-Busheri F, Rasouli-Nia A, Weinfeld M, Tomkinson AE, Izumi T, Prasad R, Wilson SH, Mitra S & Hazra TK (2004) AP Endonuclease-Independent DNA Base Excision Repair in Human Cells. *Molecular cell* **15:** 209–220

Xiao W & Samson L (1993) In vivo evidence for endogenous DNA alkylation damage as a source of spontaneous mutation in eukaryotic cells. *Proceedings of the National Academy of Sciences of the United States of America* **90:** 2117–21

Xie Y, Yang H, Cunanan C, Okamoto K, Shibata D, Pan J, Barnes DE, Lindahl T, McIlhatton M, Fishel R & Miller H (2004) Deficiencies in Mouse Myh and Ogg1 Result in Tumor Predisposition and G to T Mutations in Codon 12 of the K-Ras Oncogene in Lung Tumors. *Cancer Research* **64:** 3096–3102

Yang I, Han SJ, Kaur G, Crane C & Parsa AT (2010) The role of microglia in central nervous system immunity and glioma immunology. *Journal of clinical neuroscience* **17:** 6–10

Yang SW, Burgin AB, Huizenga BN, Robertson CA, Yao KC & Nash HA (1996) A eukaryotic enzyme that can disjoin dead-end covalent complexes between DNA and type I topoisomerases. *Proceedings of the National Academy of Sciences of the United States of America* **93:** 11534–9

Yoder KE, Espeseth A, Wang X, Fang Q, Russo MT, Lloyd RS, Hazuda D, Sobol RW & Fishel R (2011) The base excision repair pathway is required for efficient lentivirus integration. *PloS one* **6:** e17862

Zahn KE, Wallace SS & Doublié S (2011) DNA polymerases provide a canon of strategies for translesion synthesis past oxidatively generated lesions. *Current opinion in structural biology* **21:** 358–69

Zhao C, Deng W & Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. *Cell* **132:** 645–60

Zhao X, Krishnamurthy N, Burrows CJ & David SS (2010) Mutation versus repair: NEIL1 removal of hydantoin lesions in single-stranded, bulge, bubble, and duplex DNA contexts. *Biochemistry* **49:** 1658–66

Zharkov DO, Golan G, Gilboa R, Fernandes AS, Gerchman SE, Kycia JH, Rieger R a, Grollman AP & Shoham G (2002) Structural analysis of an Escherichia coli endonuclease VIII covalent reaction intermediate. *The EMBO journal* **21:** 789–800

Zharkov DO, Rieger RA, Iden CR & Grollman AP (1997) NH2-terminal proline acts as a nucleophile in the glycosylase/AP-lyase reaction catalyzed by Escherichia coli formamidopyrimidine-DNA glycosylase (Fpg) protein. *Journal of Biological Chemistry* **272:** 5335–5341

Zharkov DO, Rosenquist T a, Gerchman SE & Grollman a P (2000) Substrate specificity and reaction mechanism of murine 8-oxoguanine-DNA glycosylase. *The Journal of biological chemistry* **275:** 28607–17

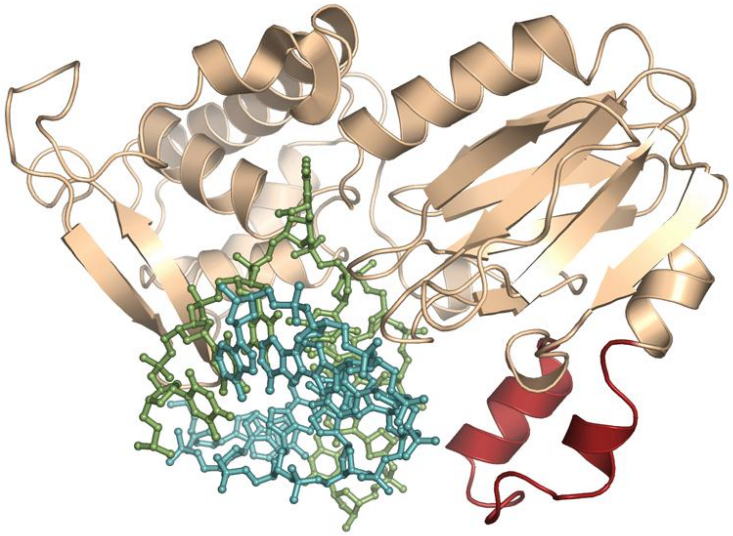
Zharkov DO, Shoham G & Grollman AP (2003) Structural characterization of the Fpg family of DNA glycosylases. *DNA Repair* **2:** 839–862

Zhou H, Xu M, Huang Q, Gates AT, Zhang XD, Castle JC, Stec E, Ferrer M, Strulovici B, Hazuda DJ & Espeseth AS (2008) Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host.Microbe* **4:** 495–504

Zhou W & Doetsch PW (1993) Effects of abasic sites and DNA single-strand breaks on prokaryotic RNA polymerases. *Proceedings of the National Academy of Sciences of the United States of America* **90:** 6601–5

ERRATA

Paper II, Figure 2b: Part of NEIL3 model is missing. Corrected figure is displayed below



Paper II, Figure 2c: ‘Leu81’ corrected to ‘Lys81’

Paper III, Figure 4, correction of numbering: a→a+b, b→c

I

II

III

IV