Audun Hanssen-Bauer

X-ray repair cross-complementing protein 1 associated multiprotein complexes in base excision repair

Thesis for the degree of Philosophiae Doctor

Trondheim, April 2012

Norwegian University of Science and Technology
Faculty of Medicine
Department of Cancer Research and Molecular Medicine

NTNU – Trondheim
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of Philosophiae Doctor

Faculty of Medicine
Department of Cancer Research and Molecular Medicine

ISBN 978-82-471-3512-9 (printed ver.)
ISBN 978-82-471-3513-6 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2012:116

Printed by NTNU-trykk

NORGES TEKNISK NATURVITENSKAPELIGE UNIVERSITET
DET MEDISINSKE FAKULTET

XRCC1 assoierte multiproteinkomplekser i base eksisjonsreparasjon

Arvestoffet (DNA) degraderes konstant av ytre faktorer, som stråling og kjemikalier, og indre faktorer, som produkter av metabolismen. Slik degradering ødelegger informasjonen som ligger i DNA, og kan derfor være toksisk for cellene og mutagent under replikasjon. Sannsynligheten for mutasjon er likevel ekstremt lav fordi DNAets integritet opprettholdes ved en lang rekke reparasjonsmekanismer. Disse involverer mange enzymer, struktur- og regulatoriske proteiner, med overlappende roller. Feil eller mangelfull reparasjon er drivkraften bak utviklingen av alderdomsrelaterte sykdommer og kreft, men er samtidig grunnlaget for genetisk variasjon og dermed for evolusjon.

Vårt arbeid har fokusert på sporet for DNA-reparasjon av skade på nukleinsyrer (byggestenene i DNA) og enkeltrådbrudd, det vil si baseeksisjonsreparasjon (BER). Forenklet foregår BER over fire steg: 1. Den skadede nukleinsyren fjernes. 2. Et trådbrudd introduseres i DNAets ryggrad. 3. Syntese av en eller flere nukleinsyrer. 4. Endene på hver sin side av trådbruddet kobles. Selv om BER kan reproduseres med kun fire enzymer i et reagensglass, er mer enn tjue andre kjente proteiner involvert. Ett av disse, XRCC1, har ingen enzymatisk aktivitet, men fungerer som et regulerende og organiserende protein gjennom interaksjon med flere av BER-proteinene som samlet bidrar til alle stegene.

Vi viser at XRCC1 fungerer som et stativ som samler en rekke BER-proteiner til store kompleks av varierende innhold. Disse BER-kompleksene interagerer med cellens replikasjonsmaskineri. Et av BER-enzymene, UNG2, interagerer direkte med XRCC1. Resultatene bekrefter hypotesen om at BER er tett knyttet til replikasjon, og avkrefter at BER drives frem av enzymers suksessive interaksjon med XRCC1 (paper 1). Sammensetningen av XRCC1-kompleksene varierer avhengig av type eller mengde skade som påføres DNA. De utvides til å inkludere proteiner involvert i replikasjon og BER-syntese av flere nukleinsyrer. Vi avkrefter hypotesen om at XRCC1-komplekser

kun kan gjennomføre den underkategori av BER som syntetiserer en enkel nukleinsyre før sammenkoblingen av trådbruddet (paper 2).

Ulike deler av XRCC1-proteinet, som er 633 aminosyrer langt, bidrar til BER. Vi viser at den sentrale regionen mellom aminosyrene 315 og 403 er nødvendig for XRCC1s evne til å samles ved DNA-skader. Regionen mellom aminosyrene 166 og 311 er med på å bestemme utstrekningen av akkumulasjonen. Vi avkrefter at XRCC1-rekruttering er avhengig av poly(ADP)ribosylering. XRCC1s bidrag til BER av metyleringsskader er ikke avhengig av dens tette interaksjon med DNA-polymerase beta og ligase 3, og regionen som interagerer med det sentrale replikasjonsorganiserende proteinet PCNA er ikke nødvendig for XRCC1-rekruttering til replikasjonsmaskineriet. De vanligste mutasjonene av XRCC1 kan føre til svekket rekruttering av XRCC1-komplekser til DNA-skader (paper 3). XRCC1s akkumulasjon til UV-induserte DNA-skader blir regulert av et signalsporet som involverer p38 mitogen aktivert kinase (MAPK). p38 MAPK er et kjent stressresponsspor for bl.a. UV-stråling og inflammasjon. Våre resultater er de første observasjonene av at dette signalssporet kan påvirke et DNA-reparasjonsspor (paper 4).

Resultatene bidrar til kunnskapen om hvordan BER organiseres og reguleres, og BER er ett av mange spor man forsøker å påvirke ved behandling av kreft. Målrettet dysregulering av slike spor har potensial for å forbedre effekten av cellegift. Våre resultater viser at å hemme poly(ADP)ribosylering ikke nødvendigvis vil ha forventet effekt på BER. Ett av de signalsporene man ønsker å påvirke for behandling av kroniske inflammasjons-sykdommer, p38 MAPK sporet, kan også påvirke BER. Selv små endringer i balansen av reguleringen av XRCC1-rekruttering kan ha betydelige effekt når en hel organisme påvirkes over lang tid.

Kandidat: Audun Hanssen-Bauer
Institutt: Institutt for Kreftforskning og Molekylærmedisin
Veileder: Marit Otterlei
Biveileder: Geir Slupphaug
Finansiering: Helse Midt-Norge, Norsk Forskningsråd (FUGEII)

Contents

Acknowledgements

The work presented in this thesis was carried out in the laboratory of Professors Marit Otterlei, Hans Krokan, and Geir Slupphaug at the Department for Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology. I am grateful for the financial support from the Central Norway Regional Health Authority, Norwegian University of Science and Technology, and the Norwegian Research Council.

Foremost I wish to express my gratitude to my supervisor Marit Otterlei for her invaluable help and guidance. I am deeply impressed by her capacity to stay up to date both theoretically, experimentally, and by her almost uncanny gift for pinpointing where an experiment turned awry. I also wish to thank all my colleagues at the "DNA repair group" who all contribute to a professionally stimulating environment while never forgetting to keep it fun and socially inclusive. I am proud to have worked with you and moved by your encouragements and trust.

Special thanks to Dr. Javier Peña-Diaz who took me under his wing, thought me how to navigate the lab, and to be a proper critic; to Dr. Karin Solvang-Garten for sharing all the joys and burdens of this thesis; to Dr. Karin Margareta Gilljam for never taking anything I say for granted; to Dr. Mansour Akbari for his encouragements, discussions and impressive commitment to our work; to Dr. Mirta Mittelstedt Lol de Sousa for keeping me sane and providing an endless supply of candy (I'm the one who kept stealing it); to Siri Bachke for making sure I got out to breathe some fresh air once a week; to Grete K. Pettersen for never losing faith in sub-cloning and blots; to Antonio Sarno for holding my hand through the last stages of this work and providing ample amounts of wonderful distractions; and to Pål Eilertsen for keeping me busy with more than science.

My gratitude also goes to my family and friends, who have never given up trying to understand what I do. I especially want to thank my dear parents for their unfaltering encouragements and love. My greatest gratitude goes to my wife and best friend Nadège. You make me smile all the way home every day!

Oslo, January 2012

List of Papers

Paper 1:

Direct interaction between XRCC1 and UNG2 facilitates rapid repair of uracil in DNA by XRCC1 complexes

Akbari, M., Solvang-Garten, K.*, Hanssen-Bauer, A.*, Lieske, N. V., Pettersen, H. S., Pettersen, G. K., Wilson, D. M. 3rd., Krokan, H. E., Otterlei, M.

*These authors contributed equally to this work

Published: DNA Repair (Amst), 2010. 9(7): p. 785-95

DOI: 10.1016/j.dnarep.2010.04.002

Paper 2:

XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage.

Hanssen-Bauer, A.*, Solvang-Garten, K.*, Sundheim, O., Pena-Diaz, J., Andersen, S., Slupphaug, G., Krokan, H. E., Wilson, D. M.3rd., Akbari, M., Otterlei, M.

* These authors contributed equally to this work

Published: Environ Mol Mutagen, 2011. 52(8): p. 623-35.

DOI: 10.1002/em.20663

Paper 3:

The region of XRCC1 from residues 310 to 436, which harbours the three most common nonsynonymous polymorphic variants, is essential for the scaffolding function of XRCC1

Hanssen-Bauer A.*, Solvang-Garten K.*, Gilljam K.M., Thorseth K., Wilson D.M. 3rd, Akbari M., Otterlei M.

*These authors contributed equally to this work

Published: DNA Repair (Amst), 2012. 11: p. 357-366
DOI: 10.1016/j.dnarep.2012.01.001

Paper 4:

p38 mitogen activated kinase mediates regulation of base excision repair / single strand break repair

Hanssen-Bauer A., Solvang-Garten K.*, Sousa M.M.L.*, Otterlei M.

*These authors contributed equally to this work

Manuscript submitted January 2012

Abbreviations

3meG	3-methyladenine
4-AN	4-amino-1,8-naphtalamide
5meC	5-methylcytosine
7meG	7-methylguanine
8-oxoG	7,8-dihydro-8-oxo-deoxyguanine
A	Adenine
aa	Amino acids
AP	Apurinic/apyrmidinic
APE1	AP-endonuclease 1
APLF	Aprataxin and PNKP like factor
APTX	Aprataxin
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3-related
BER	Base excision repair
bp	Base pairs
BRCT	BRCA1 C terminus
C	Cytosine
Cdc25(A/B/C)	Cell division cycle 25 homolog (A, B, or C)
CDK	Cyclin dependent kinase
Chk(1/2)	Checkpoint kinase (1 or 2)
CHO	Chinese hamster ovary
CK2	Casein kinase 2
CPD	Cyclobutane pyrimidine dimers
CpG	Cytosine residues followed by guanine
CtIP	CtBP interacting protein
DDR	DNA damage response

DNA	Deoxyribonucleic acid
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
dNTP	Deoxynucleotide triphosphate
dRP	3'-α,β-unsaturated aldehyde
DSB	Double strand break
DSBR	Double strand break repair
dsDNA	Double stranded DNA
ECFP	Enhanced cyan fluorescent protein
EYFP	Enhanced yellow fluorescent protein
FEN1	Flap endonuclease 1
FHA	Forkhead-associated
G	Guanine
GFP	Green fluorescent protein
H_2O_2	Hydrogen peroxide
HR	Homologous recombination
IR	Ionizing radiation
Ku70/80	ATP-dependent DNA helicase II Subunits (70 or 80 kDa)
LIG3	Ligase 3
LP	Long patch
MEF	Mouse embryonic fibroblast
mIF	micro-irradiated induced foci
MMR	Mismatch repair
MMS	Methyl methanesulfonate
MPG	N-Methylpurine DNA Glycosylase

MRE11	Meiotic recombination 11 homolog 1
MRN complex	MRE11 / RAD50 / NBS1
mtDNA	Mitochondrial DNA
NBS1	Nijmegen breakage syndrome 1
NEIL	Nei endonuclease VIII-like protein
NER	Nucleotide excision repair
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
NHEJ	Non-homologous end joining
NLS	Nucleolar localization signal
•OH	Hydroxyl radical
$\bullet O2^-$	Superoxide radical
O^6-meG	O^6-methylguanine
PAR	Poly(ADP)ribose
PARBM	Poly(ADP-ribose) binding motif
PARP(1/2)	Poly(ADP-ribosyl) polymerase (1 or 2)
PARylation	Poly(ADP-ribosyl)ation
PCNA	Proliferating cell nuclear antigen
PI3K	Phosphatidylinositol 3-kinase-like protein kinase
PJ34	N-(5,6-dihydro-6-oxo-2-phenanthridinyl)
PNKP	Polynucleotide phosphatase/kinase
POL(β/γ/δ/ε/θ/ι)	DNA polymerase (β,γ,δ,ε,θ, or ι)
PTM	Post translational modification
RAD50	Radiation repair protein RAD50
ROS	Reactive oxygen species
RPA	Replicating protein A complex
SCE	Sister chromatide exchange
SNP	Single nucleotide polymorphism

SP	Short patch
SSB(s)	Single strand break(s)
SSBR	Single strand break repair
ssDNA	Single stranded DNA
T	Thymine
TDP1	Tyrosyl-DNA phosphodiesterase 1
THF	3-hydroxy-2-hydroxymethyltetrahydrofuran
TLS	Trans-lesion synthesis
U	Uracil
UNG2	Uracil-DNA glycosylase 2
UV(A/B/C)	Ultraviolet radiation (segment A, B, or C)
XRCC1	X-ray repair cross-complementing protein 1

Introduction

The ability to store information, in form of linear combinations of bases in deoxyribonucleic acid (DNA) and transfer through generations is the most basic common trait of all living organisms. Subtle changes are introduced during the course of evolution, but as a whole the information remains remarkably stable. The five million years that separates chimpanzees and humans from their common ancestor has only introduced a ~5% divergence between their genomes (Britten, 2002). Nevertheless, DNA itself is far from stable. The human genome, with its approximately 3.2×10^9 base pairs (bp), accumulates thousands of lesions per cell per day merely by reaction with byproducts of cellular respiration, and DNA can be altered in a multitude of other ways and several kinds of lesions are produced (reviewed in (Friedberg *et al.*, 2006)). Despite the continuous and high rate of genomic degradation, the rate of accumulation of somatic mutations is as low as 10^{-6} per cell division (Araten *et al.*, 2005). DNA degradation has always been present and mechanisms to protect genomic integrity have evolved alongside DNA's success as the main molecular carrier of genetic information on Earth.

Decades after Crick, "[…]*came to realize that DNA is so precious that probably many distinct repair mechanisms would exist*" (Crick, 1974), several DNA repair mechanisms have been described, and they can be categorized into direct reversal, strand break repair, and excision repair. This thesis focuses on one of the key actors in the base excision repair / single strand break repair (BER/SSBR) pathway: X-ray repair cross-complementing protein 1 (XRCC1), and its function as a scaffolding factor and response to genomic degradation within mammalian cells. The following will therefore mainly deal with BER, XRCC1, and proteins that interact with XRCC1. However, promiscuous proteins such as XRCC1 are seldom merely involved in a single biochemical pathway since interaction with other proteins suggests involvement with many other pathways.

DNA lesions

DNA is a large and reactive macromolecule subjected to a complex and ever changing environment. DNA lesions occur spontaneously in neutral conditions, by reaction with molecules of both endogenous and exogenous origin, errors during DNA synthesis, and energy absorption from high-energy electromagnetic radiation (reviewed in (Friedberg *et al.*, 2006)).

Spontaneous damage

The N-glycosylic bonds that attach bases to the sugar-phosphate backbone of DNA are susceptible to spontaneous hydrolytic cleavage, which generates apurinic/apyrimidinic (AP) sites. AP sites are both highly mutagenic, because they leave a gap in the template strand during DNA replication, and susceptible to hydrolysis and AP endonuclease mediated excision resulting in single strand breaks (SSBs). Spontaneous depurination in human cells is estimated to be ~10 000 purines per day and the rate of depyrimidation is 20-fold lower. Another type of spontaneous hydrolytic DNA damage is deamination of bases. Loss of the exocyclic amino group of adenine (A), guanine (G), cytosine (C) or 5-methylcytosine (5meC) respectively converts them to hypoxanthine, xanthine, uracil (U), and thymine (T). Hence deamination of 5meC and C potentially leads to G:C to A:T transition mutations since both T and U pair with A. Hypoxanthine potentially pairs with G, and thus leads to A:T to G:C transition mutations, while xanthine does not stably pair with any bases, but may arrest DNA synthesis (reviewed in (Friedberg *et al.*, 2006)).

Reactive oxygen species

Reactive oxygen species (ROS), such as superoxide radical ($\bullet O_2^-$) and hydroxyl radical (•OH), damage intracellular macromolecules such as proteins, lipids, carbohydrates, and DNA. The major endogenous source of ROS is probably by-products from the electron transport chain associated with mitochondrial respiration, but ROS can also be actively produced by neutrophiles and monocytes during inflammation (reviewed in (Ohshima *et al.*, 2003; Friedberg *et al.*, 2006)). Exogenous sources include ultraviolet-, α-, β-, and γ-radiation of water, and ingestion of redox cycling compounds. The reactivity, half life,

and diffusibility of ROS influence the capacity to damage DNA. While •OH is one of the most reactive of the primary ROS, and probably the major source of oxidative damage of DNA, it is not capable of diffusing more than two molecule diameters before it reacts with endocellular molecules. However, both $•O_2^-$ and •OH are readily converted to hydrogen peroxide (H_2O_2). H_2O_2 is relatively inert with high diffusibility, and can generate •OH and other ROS in the vicinity of DNA through the Fenton reaction in the presence of Fe^{2+}. •OH mainly attacks DNA integrity by its addition to double bonds of DNA bases or by removing hydrogen from the deoxyribose backbone, resulting in a multitude of altered bases, base loss, and strand breaks. One of the most frequent relevant base alteration caused by ROS is 7,8-dihydro-8-oxo-deoxyguanine (8-oxoG). 8-oxoG has the ability to pair with A during replication and thus cause G:C to T:A transversion mutations (reviewed in (Friedberg *et al.*, 2006)).

DNA replication errors

Although DNA polymerases almost always select the correct incoming deoxynucleotide triphosphate (dNTP) during DNA synthesis, errors do occur. Misincorporation of nucleotides or slippage of the template strand cause base substitutions, deletions and additions if left unattended. However, the replicative DNA polymerases POLε, POLδ, and POLγ have 3'-5' exonuclease activity that enables them to remove mispaired or unpaired nucleotides. Proofreading through 3'-5' exonuclease activity makes the replicative the most accurate DNA polymerases with estimated error rates of less than 10^{-5} errors per synthezied nucleotide (reviewed in (McCulloch *et al.*, 2008)). Although these numbers are low, they potentially translate into several thousand mispaired bases when the whole human genome is replicated. Mismatched bases that escape DNA polymerases are repaired by the mismatch repair (MMR) pathway. In addition to errors in base pairing, DNA polymerases can also introduce lesions by incorporation of damaged nucleotide precursors, *e.g.* 8-oxo-dGTP, or dUTP (reviewed in (Friedberg *et al.*, 2006)).

DNA replication is susceptible to lesions encountered in the template strand. SSBs are converted into double strand breaks (DSBs) by the replication machinery (replication collapse), and must be repaired by doubles strand break repair (DSBR) pathways,

typically through homologous recombination (HR), before replication can be completed (reviewed in (Friedberg *et al.*, 2006)). The stringent base pairing requirement of replicative DNA polymerases causes replication arrest when they encounter DNA intra-strand crosslinks, bulky adducts and certain base lesions. However, these lesions need not always be assessed by a DNA repair pathway before replication proceeds. DNA polymerases with low base pairing stringency and no proofreading can replicate past lesions through processes collectively known as trans-lesion synthesis (TLS). The low requirement for correct base pairing greatly attenuates the fidelity of the DNA polymerases involved in TLS, and they are consequently prone to introduce errors (reviewed in (McCulloch *et al.*, 2008)).

Ionizing radiation

α-, β-, γ- and X-ray radiation may ionize atoms. Individual exposure to ionizing radiation (IR) can vary considerably, but is omnipresent. IR can both directly ionize bases and sugars in DNA and cause indirect damage through reactive species formed such as reactive oxygen species (ROS) or other molecules surrounding DNA. Thus, IR causes base modifications, AP sites, SSBs and DSBs (reviewed in (Friedberg *et al.*, 2006).

Ultraviolet radiation

The ultraviolet spectrum of electromagnetic radiation (UV; 100-400 nm) is typically subdivided into three segments; UVC (100-295 nm), UVB (295-320 nm), and UVA (320-400 nm). The sun emits radiation throughout the whole spectrum, but the composition of the UV at the Earth's surface largely depends on atmospheric properties. Oxygen in the atmosphere irradiated with wavelengths below 242 nm is converted into ozone, and ozone in turn absorbs wavelengths below 336 nm. This oxygen shield efficiently blocks UVC and attenuates UVB. At sea level solar UV irradiation constitutes of 5-10% UVB and 90-95% UVA (Ravanat *et al.*, 2001). The aromatic rings of purines and pyrimidines absorb wavelengths below 320 nm, with a collective absorption maximum at 260 nm. Thus, UVC and UVB are readily absorbed by DNA, and its excitation mainly generates intra-strand crosslinks, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidione adducts (6,4-

photoproducts). UVA is weakly absorbed by DNA, but induces oxidative DNA lesions through excitation of other endogenous chromophores that in turn generate ROS (reviewed in (Friedberg *et al.*, 2006)). Surprisingly, UVA irradiation also induces high levels of CPDs compared to the induced ROS lesions (Douki *et al.*, 2003; Mouret *et al.*, 2006). The mechanisms behind UVA induced CPDs are still debated and energy transfer to DNA both through direct absorption and indirectly through photosensitizers has been suggested (reviewed in (Girard *et al.*, 2011)). However, broad-band UVB (295-320 nm) induces 1000-fold more CPDs than broad-band UVA (340-400 nm) in Chinese hamster cells (Douki *et al.*, 2003). The distribution of lesion types induced by irradiation with different wavelengths within the UV range still needs further research, but as a whole, mediation of intra-strand crosslinking seems to decrease and ROS lesions increase with increasing wavelengths within the UV spectrum. Moreover, the lesion distribution is also influenced by lesion intermediates generated by excision repair pathways and dose-dependent clustering of lesions.

A major advance in the understanding of DNA repair responses has come from the development of microirradiation techniques in living cells and the visualization of responses by immunohistochemistry or fluorescent tags and microscopy (reviewed in (Nagy *et al.*, 2009)). Upon irradiation of delimited areas of nuclei with IR or UV (microirradiation), DNA repair proteins accumulate in the irradiated area. The accumulation is seen as bright spots of variable intensity commonly referred to as foci. Relative quantification of foci intensities and/or photobleaching techniques provide information about the dynamics of the studied factors. The radiation source and dose and cellular pretreatment with photosensitizers largely determine DNA lesion distribution and consequently the selection of recruited repair factors (Kong *et al.*, 2009).

Alkylating agents

A broad range of endogenous and exogenous chemicals have the capacity of damaging DNA. The types of lesions include alkylation, crosslinking, intercalation, covalent attachment, and strand breaks, but only alkylation will be discussed here.

The most common alkylation of DNA is methyl transferase-directed methylation of C residues followed by G (CpG islands). Between 60% and 80% of all CpG islands in mammals contain 5meC (Ehrlich *et al.*, 1982). Methylation of CpG islands in the promoter region of genes is involved in regulation of gene expression, and contributes cellular differentiation and epigenetic regulation (Jaenisch *et al.*, 2003).

Alkylating agents can be either mono- or bi-functional. Monofunctional agents interact covalently with one nucleophilic center, while the bi-functional have the capacity to interact with two. The latter thus has the potential to crosslink DNA strands. Nucleotides can be alkylated at several different nitrogen and oxygen positions, and the biological consequences are diverse (reviewed in (Friedberg *et al.*, 2006)). The most abundant endogenous methyl donor S-adenosylmethionine has been estimated to produce 4000 7-methylguanines (7meG), 600 3-methyladenines (3meA) and 10-30 O^6-methylguanines (O^6-meG) per human cell per day (Rydberg *et al.*, 1982). 7meG does not influence base pairing or the replication machinery and is therefore considered quite harmless (Marnett *et al.*, 1993). However, 7meG are unstable and spontaneously degrade to AP sites (reviewed in (Shrivastav *et al.*, 2010)). 3meA blocks replication and is highly toxic (Karran *et al.*, 1980; Evensen *et al.*, 1982). O^6-meG is less prevalent than both 7meG and 3meA, but is both cytotoxic and prone to cause G:C to A:T transition mutations because the methylation interferes with base pairing (Povey *et al.*, 2002).

Exogenous alkylating agents are numerous and exposure varies considerably. For example, smoking exposes lung tissue to a variety of nitrosamines that alkylate DNA, but nitrosamines can also be found in food, beverages, and even potable water (Scanlan, 1983; Hoffmann *et al.*, 1985; Brunnemann *et al.*, 1987; Moller *et al.*, 1989).

Consequences of DNA damage

The consequences of DNA lesions vary from none to lethal depending on the type of lesion, where it is located in the genome, cell type, and cell cycle state. Lesions that do not alter DNA structure or replication, or are outside of regulatory and coding regions are less likely to be deleterious. Conversely, a single lesion in a vital coding or

regulatory region can potentially cause cell death or trigger dysregulation of cellular processes leading to morbidity.

Lesions in gene sequences commonly block transcription, rendering the gene products inaccessible or cause complementation errors. Lesions encountered during DNA replication are particularly challenging since error-free DNA synthesis requires strand dissociation and an intact template strand. A cell's genomic state, and its intra- and extra-cellular environments are continuously monitored and tightly connected to regulation of DNA repair, cell cycle, and apoptosis (reviewed in (Warmerdam *et al.*, 2010)). DNA repair counters genomic degradation, and when accumulation of lesions above normal levels occurs, proliferating cells can arrest cell cycle progression to allow for further DNA repair. If the genomic degradation is not sufficiently countered by cell cycle arrest and subsequent DNA repair cells of multicellular organisms go into apoptosis. Several hundred kinase substrates involved in almost every significant cellular process are phosphorylated in response to DNA damage (Matsuoka *et al.*, 2007; Stokes *et al.*, 2007). Thus, our knowledge of the coordination of the cellular responses to genomic degradation is limited and observations hard to interpret as they involve convergence of many signaling pathways and regulatory mechanisms.

DNA lesion detection

Eukaryotic DNA is organized as coils around cores of histones forming nucleosomes. Nucleosomes are comprised of eight histones forming a bead on which a 147-bp long stretch of DNA wraps around twice. Each nucleosome is separated by a stretch of at least 20 bp of DNA, and the histones of different nucleosomes can interact to fold the nucleosome string into shorter and thicker filaments known as chromatin. Chromatin is packed more or less tightly depending on the post translational modification (PTM) of histones and methylation of CpG islands. Tightly packed chromatin (heterochromatin) renders DNA inaccessible for most proteins and thus works as a mechanism for differentiation. Conversely, actively transcribed regions of the genome are organized as relaxed chromatin structures (euchromatin) that allow access to all involved proteins. Chromatin organization influences DNA susceptibility, and DNA repair is tightly connected to chromatin reorganization (reviewed in (Cann *et al.*, 2011)).

The research focus on lesions induced by highly toxic/mutagenic DNA damaging agents *e.g.* IR, UVC, and cancer drugs has created a bias in the DNA damage response (DDR) field towards research on lesions that cause severe DNA helix distortions (strand breaks, bulky adducts, and DNA crosslinks). Bulky adducts and inter- and intra- DNA strand crosslinks cause kinks in the DNA helix that are recognized by Xeroderma pigmentosum group C, and damage DNA binding protein 1. Once bound, they initiate the nucleotide excision repair (NER) pathway. Lesion detection is also coupled to transcription since stalling of RNA polymerase II triggers NER (reviewed in (Nouspikel, 2009)). Poly(ADP-ribosyl) polymerase (PARP) family members PARP1 and PARP2 both bind to, and are activated by, SSBs, and PARP1 additionally binds DSBs (de Murcia *et al.*, 1994; Ame *et al.*, 1999; Wang *et al.*, 2006). Upon activation, PARP1 and PARP2 poly(ADP-ribosyl)ate themselves and other nuclear proteins, and mediate *e.g.* chromatin relaxation, DNA repair, and regulation of cell cycle and apoptosis (reviewed in (Schreiber *et al.*, 2006)). A third member of the PARP family, PARP3, has recently been showed to be activated by DSB and mediate NHEJ (Rulten *et al.*, 2011). DSBs are also recognized by the Ku heterodimer (Ku70/Ku80) and RAD50 of the MRN complex (MRE11/RAD50/NBS1). Together these DSB detectors compete and recruit genome integrity sensors that mediate different double strand break repair (DSBR) pathways (Hochegger *et al.*, 2006; Wang *et al.*, 2006; Haince *et al.*, 2007).

Less is known about detection of lesions that do not cause significant DNA helix distortion, *e.g.* base lesions. DNA glycosylases are able to bind and process a wide variety of base lesions. Based on the potential reduction of diffusion complexity and results observed with naked DNA strands *in vitro,* DNA glycosylase lesion detection is believed to happen through an electrostatically guided migration along the DNA backbone (reviewed in (Friedman *et al.*, 2010)). The heterogenous chromatin organization and the diverging consequences of different base lesions in the heterochromatic regions of non-proliferating vs. proliferating cells oppose a hypothesis of base lesion detection merely through continuous scanning of the whole genome by independent DNA glycosylases. DNA replication on the other hand, represents a “once in a cell cycle” event in which chromatin is both relaxed and the whole interacts with

DNA processing enzymes that depend on DNA integrity. Proliferating cell nuclear antigen (PCNA), *e.g.* a scaffold protein in replication, has been shown to interact with several DNA glycosylases and other factors vital for BER, NER, and MMR (reviewed in (Moldovan *et al.*, 2007)).

Genome integrity sensors

Genome integrity is continuously assessed by cells and linked to the regulation of several central cellular processes, such as cell cycle, apoptosis, transcription and DNA repair pathways. As previously mentioned, mainly responses to severe DNA helix distortions have been described. However, this bias might be biologically relevant. Lesions that cause little or no DNA distortion are frequent events that commonly are transiently converted into strand breaks during repair, and potentially cause replication collapse or block respectively converting the lesion into DSB or causing accumulation of ssDNA. Sensors that are activated by severe DNA helix distortions would thus indirectly sense lesions that do not cause significant helix distortion (reviewed in (Durocher, 2009)). DDR is believed to be primarily mediated by members of the phosphatidylinositol 3-kinase-like protein kinase (PI3K) family, Ataxia telangiectasia mutated (ATM), Ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), as well as by members of the PARP family (reviewed in (Ciccia *et al.*, 2010))

Ku heterodimers bound to DSB recruit and activate DNA-PKcs which in turn initiates DSBR through non-homologous end joining (NHEJ) (reviewed in (Mahaney *et al.*, 2009)). The MRN complex bound to DSB recruits and activates ATM (Falck *et al.*, 2005; Berkovich *et al.*, 2007). Once activated, ATM regulates resection of DSB termini through CtBP interacting protein (CtIP) and generates the ssDNA overhangs that are used in the strand invasion of the HR DSBR pathway during S/G2 (Jazayeri *et al.*, 2006; Shiotani *et al.*, 2009). ssDNA from either resected DSB or generated by helicases when replication is blocked is rapidly detected and coated by replicating protein A complexes (RPA; *i.e.* RPA1/RPA2/RPA3). RPA stabilizes the ssDNA and serves as a loading platform for ATR and its cofactor ATR-interacting protein (Zou *et al.*, 2003). Thus, DNA replication and DNA repair intermediate monitoring partially converges through

PI3Ks. Although ATM is believed to be mainly a DSB sensor while ATR senses replication stress, their functional distinction is far from absolute. As mentioned, ATR senses resection of DSBs, and collapsed replication results in a DSB that is in turn sensed by ATM. Furthermore, many ATM and ATR substrates overlap. Because merely ATM and ATR have over 700 putative substrates in humans, involved in almost every aspect of the biological processes of a cell (Matsuoka *et al.*, 2007), a presentation of PI3K-mediated DDR is beyond the scope of this thesis. ATM and ATR mediate cell cycle arrest mainly through their respective substrates, the checkpoint kinases (Chk) Chk1 and Chk2. Activated Chk1 and Chk2 phopshorylate and inactivate the cell division cycle 25 homologs A, B and C (Cdc25A, Cdc25B, Cdc25C). The inactive Cdc25 homologs cannot activate the cyclin dependent kinases (CDKs) that drive cell cycle through G1/S-, S-, or G2/M-phases of the cell cycle. Phosphorylation by PI3Ks and Chk2 also mediate activation of the tumour suppression transcription factor p53. Among its many targets, p53 can activate expression of members of the Bcl-2 protein family that trigger apoptosis, and p21 which inhibits CDKs and mediates G1/S or S-phase arrest (reviewed in (Sakasai *et al.*, 2009)).

Poly(ADP-ribosyl) polymerase 1 and 2 and SSB detection

Among the 17 members of the the PARP family of proteins only five are considered true PARPs (able to transfer ADP-ribose moieties to acceptor proteins) and among these, PARP1, PARP2, and PARP3 has so far been observed to respond to DNA strand breaks ((Ame *et al.*, 1999; Iles *et al.*, 2007) and reviewed in (Rouleau *et al.*, 2010)). PARP1, PARP2 and PARP3 mediate DDRs through protein interactions, mono(ADP-ribosyl)ation and poly(ADP-ribosyl)ation (PARylation). Of the three most is known about PARP1, which has been linked to many cellular processes such as DNA repair, chromatin organization, transcription, cell cycle regulation, and inflammation (reviewed in (Rouleau *et al.*, 2010)). PARP2 is expected to share many of these functions, and similarities have been observed (reviewed in (Schreiber *et al.*, 2006)). PARP1 and PARP2 both form homodimers, but are also found as heterodimers (Bauer *et al.*, 1990; Schreiber *et al.*, 2002). Both become activated when bound to SSBs, directly induced or as BER intermediates, and share similar expression patterns and interact with the BER scaffolding factor XRCC1 (Caldecott *et al.*, 1996; Schreiber *et al.*, 2002). However,

mice or cells deficient in either PARP1 or PARP2 show diverging phenotypes, and the protein interactions of PARP1 and PARP2 in human lysates only partially overlap (Yelamos *et al.*, 2008; Isabelle *et al.*, 2010). In response to UV, ROS, or alkylating agents, PARP1 is responsible for 90% of observed PARylation activity (Ame *et al.*, 1999; Schreiber *et al.*, 2002).

Upon activation, PARP1 and PARP2 start assembling branched chains of up to 200 ADP-ribose moieties on themselves and many other targets. Poly(ADP)ribose (PAR) chains are rapidly disassembled by PAR glycohydrolase, making PARylation a transient event that last only minutes (reviewed in(Okano *et al.*, 2003)). The ADP-ribose units contain an A moiety capable of forming hydrogen bonds and carry two negatively charged phosphate groups. PAR chains thus potentially cause both steric and noncovalent interactions with other molecules (reviewed in (D'Amours *et al.*, 1999)). PARylation of histones in response to DNA damage contributes to chromatin reorganization, and PAR chains mediate DNA repair and signaling of the genomic state (reviewed in (Schreiber *et al.*, 2006)). PAR also serves as a platform that recruits proteins. Three PAR association motifs have been described: a macrodomain, a PAR-binding Zinc finger motif, and a cluster of 8 amino acids (aa) rich in acidic and hydrophobic residues (refered to as PAR binding motif; PARBM). Together these PAR association domains interact with more than 300 proteins, among which DDR and DNA repair proteins are overrepresented in addition to many DNA replication and transcription factors (Gagne *et al.*, 2008). Putative PARBM have been identified in XRCC1 and Ligase 3α (LIG3α) (Pleschke *et al.*, 2000).

When monitoring responses to strand breaks induced by near-UVA, both PARP1 and PARP2 accumulate to microirradiation induced foci (mIF). However, while PARP1 is rapidly and transiently accumulated, PARP2 accumulation is slower and lasts longer (Mortusewicz *et al.*, 2007). The delayed PARP2 recruitment compared to PARP1 indicates distinct roles in DNA repair. Interestingly, accumulation of both PARP1 and PARP2 in near-UVA microirradiated areas where enhanced by PARP1 activity (Mortusewicz *et al.*, 2007). A central role of PARP1 was also observed for XRCC1 recruitment. While PARP1 deficiency was associated with a considerable reduction of

XRCC1 recruitment to near-UVA mIF, PARP2 deficiency was not (Mortusewicz *et al.*, 2007). A similar reduction of XRCC1 recruitment to near-UVA mIF was observed when inhibiting PARylation (Godon *et al.*, 2008). Together these observations indicate that XRCC1 recruitment to DNA damage depends on PARP1 activity.

As SSB sensors, PARP1 and PARP2 initiate SSBR by mediating recruitment of XRCC1/ LIG3α/DNA Polymerase β (POLβ) and associated BER/SSBR factors. LIG3α interaction with both PARP1 and PAR has been observed to stimulate the ligation step (Leppard *et al.*, 2003). However, while PARP1 knock-down reduces SSB rejoining in G1-phase, no significant reduction is observed in S-phase. This indicates SSBR redundancy by S-phase-specific repair pathways (Godon *et al.*, 2008). BER is initiated by glycosylases and PARP1/PARP2 activation likely follows base removal (figure 1. step II). PARP1 has a high affinity for stalled BER intermediates and promotes gap filling past blocked 5' termini by strand displacement (long patch BER, discussed later) (Dantzer *et al.*, 2000; Lavrik *et al.*, 2001; Prasad *et al.*, 2001). However, PARP1 has recently also been shown to bind AP sites and has been proposed to protect lesions pending strand incision by AP endonuclease 1 (APE1) or 5'-dRP lyase activity (Khodyreva *et al.*, 2010). Both PARP1 and PARP2 deficient MEF show reduced repair capacity of lesions induced by alkylation, and PARP1/PARP2 double knockout mice are not viable, indicating mutual dependency (Schreiber *et al.*, 2002; Menissier de Murcia *et al.*, 2003).

Base excision repair

More than 20 proteins have been shown to be involved in human BER, but merely four are necessary to reproduce the BER pathway *in vitro* (Kubota *et al.*, 1996). BER can be presented as a core pathway of five enzymatic steps although the *in vivo* situation is much more complex (figure 1. steps I to V).

BER is initiated when a base lesion is recognized by a glycosylase. The glycosylase flips the nucleotide, placing the base in an active pocket, and hydrolyzes the N-glycosylic bond that attaches the base to the deoxyribose leaving an AP site (figure 1. step I). This specific removal of lesions as free bases distinguishes BER from other types of repair. The phosphodiester bond of AP sites is then hydrolyzed by enzymes with AP endonuclease activity, producing a gap in the double stranded DNA (dsDNA) (figure 1. step II). Gaps with 3' hydroxyl termini allows DNA polymerases to initiate DNA synthesis, referred to as gap filling (figure 1. step III), and 5' termini are trimmed to 5' phosphate (figure 1. step IV) allowing ligation by ligases (figure 1. step V). The production of AP sites and gapped dsDNA as repair intermediates, and the involvement of several 3' and 5' termini tailoring enzymes, make BER a proficient repair pathway for spontaneously induced AP-sites and SSB, and exo- and endogenously induced SSB (*e.g.* by ROS and IR).

BER in mitochondria

Mitochondria are the only eukaryotic organelles apart from the nucleus that contain DNA. The emplacement of mitochondrial DNA (mtDNA) puts it in proximity of the main source of endogenous ROS source and its mutation rates in mammals are 10 to 200-fold higher than nuclear DNA (Pesole *et al.*, 1999). mtDNA is a closed-circular molecule of approximately 16,6 kb encompassing 37 genes, which codes for 13 of the approximately 90 proteins that are involved in the electron transport chain of respiration (reviewed in (Friedberg *et al.*, 2006)). The importance of maintenance of mtDNA integrity is apparent as mutations are associated with ageing, cancer and hereditary diseases (reviewed in (Park *et al.*, 2011)). Base lesions in mtDNA are processed through the same five enzymatic steps as described for nuclear BER. The presence of BER factors depends on mitochondrial import, and, in many cases, mitochondrial and nuclear

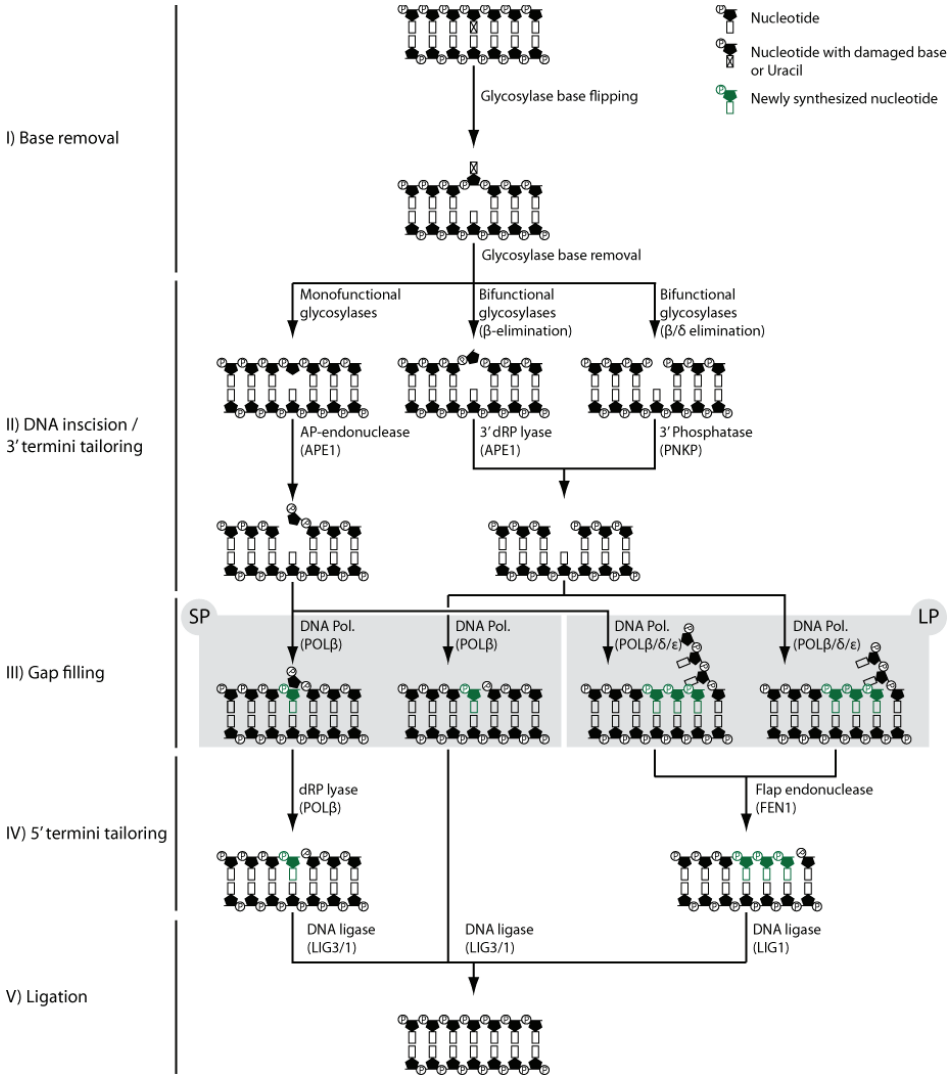


Figure 1. The base excision repair pathway

I) to V) core enzymatic steps. Base removal (I) is shared by all DNA glycosylases, while they vary in their DNA incision (II). Gap filling is divided in two subpathways (III), were DNA polymerases synthesize either a single, short patch (SP), or several, long patch (LP), nucleotides. 5' termini are processed (IV) before ligation (V).

BER factors are isoforms generated by alternative splicing (*e.g. Ung* and *Lig3*) (Nilsen *et al.*, 1997; Lakshmipathy *et al.*, 1999). Mitochondrial BER seems to rely on simpler complexes than nuclear BER. Several nuclear BER factors are not present in mitochondira, *e.g.* the scaffolding factor XRCC1 and probably FEN1, and seem to contain only one DNA polymerase (POLγ) (Lakshmipathy *et al.*, 2000; Kaguni, 2004; Akbari *et al.*, 2007; Akbari *et al.*, 2008; Szczesny *et al.*, 2008).

BER scaffolding

In 1990, Thompson *et al*. discovered the first mammalian gene that affects cellular sensitivity to IR, naming it X-ray cross complementing 1 (Thompson *et al.*, 1990). Xrcc1-deficient Chinese hamster ovary (CHO) cell lines have ~10-fold increased sensitivity to monofunctional alkylating agents, ~2-fold increased sensitivity to strand breaks and ROS mediating agents, and a ~10-fold increase in sister chromatide exchange (SCE) (reviewed in (Thompson *et al.*, 2000; Caldecott, 2003)). The hypersensitivity of XRCC1-deficient cells to base lesions and strand breaks is a result of severely impaired BER/SSBR efficiency (reviewed in (Brem *et al.*, 2005)). Despite the effect on BER/SSBR, XRCC1 has no known enzymatic activity. However, XRCC1 has been observed to interact with and stimulate a multitude of DNA repair factors within the BER/SSBR pathway, *i.e.* DNA glycosylases, SSB termini trimming factors, DNA polymerases, and ligases, and is thus believed to work as a BER/SSBR scaffolding factor. XRCC1 homologs seem to be restricted to eukaryotes with genomes larger than that of the social amoeba *Dictyostelium* (34 Mb), indicating a role as a mediator of increased efficiency and/or regulation of BER/SSBR (Caldecott, 2008). Unaddressed strand breaks and base lesions or BER/SSBR intermediates encountered during DNA replication cause replication block or collapse, triggering DSBR pathways and TLS. Dependency on recombinational repair and error-prone polymerases could in part explain the increased genomic instability of XRCC1-deficient cells. However, there are indications that XRCC1-deficient cells also show reduced repair of IR induced DSBs indicating involvement in DSBR (reviewed in (Caldecott, 2003)). Accordingly, XRCC1 and interacting factors have been shown to participate in DNA-PK independent NHEJ (alternative NHEJ) (Audebert *et al.*, 2004; Audebert *et al.*, 2006).

X-ray repair cross-complementing protein 1

XRCC1 is a 633 aa long protein (70 kDa) with three domains: an N-terminal domain (aa 1-183) followed by a 131 aa long region containing a nuclear localization signal (NLS), and two BRCA1 C terminus (BRCT) domains (BRCT1: aa 315-403, BRCT2: aa 538-633) separated by 134 aa (inter BRCT region) (figure 2. B)

The N-terminal domain of XRCC1 has been shown to bind nicked and gapped DNA *in vitro,* indicating that XRCC1 may serve as a strand break sensor/anchor (Marintchev *et al.*, 1999; Mani *et al.*, 2004). However, the N-terminal domain of XRCC1 also binds POLβ with high affinity, possibly through interaction with the thumb domain of POLβ in a manner that excludes interaction with DNA (Caldecott *et al.*, 1996; Kubota *et al.*, 1996; Cuneo *et al.*, 2010). The interaction with XRCC1 stabilizes the expression of POLβ, and is required for POLβ recruitment to DNA damage (Parsons *et al.*, 2008; Hanssen-Bauer *et al.*, 2011).

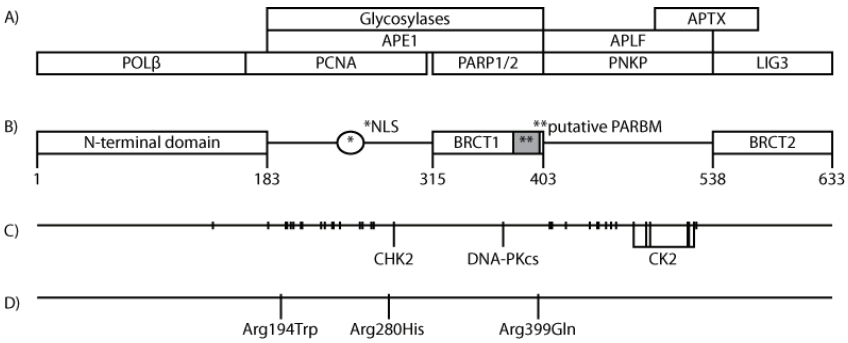


Figure 2. Schematic overview of XRCC1

A) Approximate XRCC1 protein interaction regions. B) XRCC1 domains. C) Known XRCC1 phosphorylations and phosphorylating kinases (Chk2: Thr284, DNA-PKcs: Ser371, CK2: cluster of six residues from Ser475 to Ser523, possibly more). D) Three most prevalent XRCC1 polymorphisms.

XRCC1 is translocated to the nucleus by a NLS ranging from aa 239 to 266 in the region linking the N terminal domain to BRCT1 (NLS region, aa 184-314) (Thompson *et al.*, 1990; Masson *et al.*, 1998). The NLS region interacts with PCNA sequestering a fraction of XRCC1 and its partners to the replication machinery during S-phase (Fan *et al.*, 2004).

Interaction with several DNA glycosylases (*e.g.* Uracil-DNA glycosylase 2; UNG2) is clustered in the region spanning from the NLS region to BRCT1 (aa 184-403), which also is suspected to bind APE1 (Vidal *et al.*, 2001; Marsin *et al.*, 2003; Campalans *et al.*, 2005; Akbari *et al.*, 2010). Hypoxanthine repair activity assays indicate that N-Methylpurine DNA Glycosylase (MPG) binds within the aa 1-403 region of XRCC1, although this was not confirmed by immunoblotting (Campalans *et al.*, 2005).

Of the two XRCC1 BRCT domains, BRCT1 is the most evolutionary conserved and is required for efficient DNA damage repair and proliferation after methylation damage (Taylor *et al.*, 2002; Kubota *et al.*, 2003). XRCC1 BRCT1 interacts with the BRCT domain of PARP1, and with PARP2, and encompasses a putative PARBM (aa 379-400) (Masson *et al.*, 1998; Pleschke *et al.*, 2000; Schreiber *et al.*, 2002). XRCC1 BRCT2 binds the BRCT domain of Ligase 3α (LIG3α) (Nash *et al.*, 1997; Taylor *et al.*, 1998) and, similar to XRCC1 interaction with POLβ, this interaction stabilizes the expression levels of LIG3α (Caldecott *et al.*, 1995). The XRCC1 BRCT domains also serve as inter-XRCC1 interaction modules, although their respective contribution is still somewhat unclear. Beernink *et al.* reported in 2005 that XRCC1 BRCT1 domains could form a heterotetrameric interaction with PARP1, while BRCT2 domains could dimerize directly (Beernink *et al.*, 2005). In 2006, Lévy *et al.* reported that XRCC1 dimerization was formed through BRCT1 domains and not BRCT2 (Levy *et al.*, 2006). In 2011, Cuneo *et al.* reported a crystallographically resolved structure of a XRCC1/ LIG3α tetramer with interaction between the XRCC1 BRCT2 domains (Cuneo *et al.*, 2011). Although the details on how XRCC1 forms multimers are diverging, their presence has been confirmed *in vivo* by FRET (Fan *et al.*, 2004).

The main PTM of BER proteins observed is phosphorylations (Almeida *et al.*, 2007). XRCC1 is an heavily phosphorylated protein with more than 30 known[1] phosphorylated Ser/Thr residues (Kubota *et al.*, 2003). Ser371 within BRCT1 has been shown to be phosphorylated *in vivo* by DNA-PKcs upon IR induced DNA damage. *In vitro* phosphorylation of Ser371 causes XRCC1 dimer dissociation (Levy *et al.*, 2006). However, all other verified phosphorylations of XRCC1 cluster outside of the BRCT domains, mainly within the regions between the N-terminal domain and BRCT1 (aa 183-315), and in the inter BRCT region (aa 403-538) (figure 2. C). Chk2, activated by the PI3K ATM, complexes with XRCC1 and phosphorylates Thr284. Thr284Ala mutated XRCC1 was linked to accumulation of BER intermediates possibly through modification of XRCC1s interaction with glycosylases (Chou *et al.*, 2008). Of the known kinase interactions with XRCC1, casein kinase 2 (CK2) is the most extensively documented. CK2 is a pleiotropic, ubiquitous, constitutively active kinase, involved with *e.g.* cellular growth and suppression of apoptosis. CK2 phosphorylates hundreds of different substrates and among them several factors known to be associated with cancerogenesis, such as nuclear factor kappa light chain enhancer of activated B cells (NF-κB). CK2 expression is upregulated in a wide variety of human cancers and has become a popular target for drug design (reviewed in (Hanif *et al.*, 2010)). The inter BRCT region of XRCC1 encompasses eight primary and five atypical consensus sites for CK2, and the cluster is readily phosphorylated by CK2 *in vitro* (Loizou *et al.*, 2004). Within the cluster, nine residues were observed to be phosphorylated *in vivo* by mass spectroscopy, and among them six residues close to BRCT2 showed reduced phosphorylation when CK2 is knocked down (figure 2. C) (Loizou *et al.*, 2004; Luo *et al.*, 2004). Phosphorylations of residues in the inter BRCT region stimulate binding to the forkhead-associated (FHA) domains of the SSB/DSB termini trimming factors Aprataxin (APTX), Polynucleotide kinase/phosphatase (PNKP), and Aprataxin and PNKP like factor (APLF) (Date *et al.*, 2004; Loizou *et al.*, 2004; Luo *et al.*, 2004; Bekker-Jensen *et al.*, 2007; Iles *et al.*, 2007; Kanno *et al.*, 2007). FHA domains are involved in protein/protein interactions through phospho-threonine binding, and are

[1] PhosphoSitePlus® database, search term "XRCC1"; p18887,
http://www.phosphosite.org
Last accessed 15th July 2011

found in more than 700 eukaryotic proteins such as kinases, phosphatases, kinesins, transcription factors, RNA binding proteins and metabolic enzymes (Hofmann *et al.*, 1995). Interaction with XRCC1 stimulates both the phosphatase and kinase activities of PNKP (Whitehouse *et al.*, 2001).

Although XRCC1-deficient cancer cell lines are viable, XRCC1-deficiency in mice is embryonically lethal (Tebbs *et al.*, 1999). Because XRCC1-deficient embryos die at around the seventh day, the exact physiological role of XRCC1 in foetal development is difficult to address, but the arrested embryos resemble those associated with APE1 deficiency and die at approximately the stage as *Lig3*$^{-/-}$ mice embryos (Xanthoudakis *et al.*, 1996; Stucki *et al.*, 1998; Puebla-Osorio *et al.*, 2006). Similar to the XRCC1-deficient CHO cells, XRCC1-deficient mice embryos show hypersensitivity to mutagens and increased SCE (Tebbs *et al.*, 1999). Transgene complementation in the *Xrcc1*$^{-/-}$ mice expressing less than 10% of normal XRCC1 levels is sufficient to rescue the embryonical development and produce healthy fertile adults (Tebbs *et al.*, 2003). However, heterozygous *Xrcc1*$^{+/-}$ mice, expressing 50% of normal XRCC1 levels, show increased precancerous lesions in the colon, and liver toxicity upon ingestion of alkylating agents (McNeill *et al.*, 2011).

XRCC1's central role in BER/SSBR has prompted a multitude of epidemiologic studies of XRCC1 polymorphisms[2]. The three most prevalent non-synonymous single nucleotide polymorphisms (SNPs) of *Xrcc1* lead to Arg194Trp (rs1799782), Arg280His (rs25489), and Arg399Gln (rs25487) variants in XRCC1. These SNPs have been extensively studied in relation to several types of cancer, but metastudies of these epidemiological results have so far not yielded any unambiguous relationship to cancer prevalence (Chen *et al.*, 2011; Gsur *et al.*, 2011; Huang *et al.*, 2011; Xue *et al.*, 2011). This is probably a result of both the size of populations included in the specific epidemiological studies and significant differences in the genomic SNP distribution

[2] ~60% of all articles in the Pubmed database concerning XRCC1 are epidemiological studies of XRCC1 polymorphisms. Search term "XRCC1".
http://www.ncbi.nlm.nih.gov/pubmed
Last accessed 18th July 2011

between studied populations. Homozygous Arg280His has *e.g.* been observed to be associated with breast cancer in Asians, but not in Caucasians (Li *et al.*, 2009). Homozygous Arg194Trp increases lung cancer risk in Asians, whereas heterozygous Arg194Trp in Caucasians reduces the risk (Jiang *et al.*, 2010). The only SNP considered to present "moderate amount of evidence" in relation to cancer is the -77T<C (rs3213245) XRCC1 promoter polymorphism in correlation with lung cancer (Vineis *et al.*, 2009).

DNA glycosylases

To date, eleven DNA glycosylase genes have been identified in humans (Wood *et al.*, 2005)[3]. Although the overall folding architectures of the proteins they encode are similar, their substrate specificities are generally different. DNA glycosylases recognize a wide variety of base lesions that are generated by the thousands per genome per day, but the mechanism of their lesion detection is largely unknown (reviewed in (Friedman *et al.*, 2010)). DNA glycosylases share a common lesion processing step involving DNA helix distortion that causes the damaged base to flip into a specific pocket in the active site of the DNA glycosylase. The damaged base is then released from the DNA backbone by hydrolysis of the N-glycosylic bond leaving an AP site in the DNA ((Slupphaug *et al.*, 1996) and reviewed in (Friedman *et al.*, 2010)). The following step represents the first branching point from the core BER pathway. Monofunctional DNA glycosylases do not process the AP site further, while bifunctional DNA glycosylases also have an AP lyase activity, which cleaves the DNA backbone (reviewed in (Robertson *et al.*, 2009)) (figure 1. step II). The AP lyase activity (β-elimination) of bifunctional DNA glycosylases produce a SSB with 3'-α,β-unsaturated aldehyde (dRP) and 5'-phosphate termini. The Nei endonuclease VIII-like (NEIL) family of bifunctional DNA glycosylases can further process the 3'-dRP termini to a 3'-phosphate by δ-elimination (Bandaru *et al.*, 2002; Hazra *et al.*, 2002a; Hazra *et al.*, 2002b).

[3] The URL cited in Wood et al. 2005 is incorrect. An updated table based on this article is available at: http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html#Human DNA Repair Genes Last accessed 10th October 2011.

Uracil-DNA glycosylase 2

Human *Ung* was the first DNA glycosylase gene found to encode isoforms localized either in the nucleus (UNG2) or in mitochondria (UNG1) (Nilsen *et al.*, 1997). UNG2 removes U from U:A and U:G mispairs *in vitro* and likely *in vivo* (Kavli *et al.*, 2002; Akbari *et al.*, 2004). UNG2 interacts directly with both the scaffolding factors PCNA and XRCC1, and thus co-immunoprecipitates with several replication and base lesion repair factors (Otterlei *et al.*, 1999; Akbari *et al.*, 2004; Akbari *et al.*, 2010). UNG2 has been shown to be responsible for a post-replicative removal of misincorporated dUMP (Otterlei *et al.*, 1999). This is further supported by the observation that UNG2 expression is upregulated during DNA replication (from late G1 to mid-S-phase) (Hagen *et al.*, 2008). In addition to BER, UNG2 is involved in the immune response of both mice and humans (Nilsen *et al.*, 2000; Rada *et al.*, 2002; Imai *et al.*, 2003; Kavli *et al.*, 2005)

AP site incision and 3' terminus tailoring

AP endonucleases interact with AP sites, produced either by DNA glycosylases or spontaneous base loss, by hydrolyzing the phosphodiester bond 5' of the AP site. Of the two known human AP endonucleases (APE1 and APE2), APE1 accounts for more than 95% of the AP endonuclease activity in human cells (Wilson *et al.*, 1995; Suh *et al.*, 1997). The contribution of APE2 to BER is not known and, if present, probably is very limited and difficult to assess because its AP endonuclease activity is low and APE1-independent BER has been proposed to work through both the δ-elimination of bifunctional glycosylases and Tyrosyl-DNA phosphodiesterase 1 (TDP1) (Wiederhold *et al.*, 2004; Burkovics *et al.*, 2006; Lebedeva *et al.*, 2011). Initiation of DNA synthesis requires 3' hydroxyl termini. Thus, the 3'-dRP or 3'-phosphate termini produced by bifunctional glycosylases require tailoring. Furthermore, directly induced SSBs typically do not result in 3'-hydroxyl termini. The majority of ROS induced SSBs have either 3'-phosphate or 3'phosphoglycolate termini, and abortive dsDNA unwinding by Topoisomerase1 (TOP1) causes SSB with TOP1 irreversibly bound to the 3' termini (3'-TOP1). 3'-phospate and 3' phosphoglycolate are processed by PNKP and APE1 respectively, and 3'-TOP1 by TDP1 (reviewed in (Caldecott, 2008)).

Unprocessed SSB cause replication collapse and are assessed by HR during S-phase. However, quiescent cells depend more on SSBR to resolve SSB. This might be especially relevant concerning neurons, because the nervous system has a high energy metabolism through respiration but low levels of antioxidants. Mutations in TDP1 and APTX (involved in processing 5' termini of aborted ligation and possibly 3' phosphoglycolate and 3' phosphate) have been associated to hereditary neurodegenerative diseases (reviewed in (Caldecott, 2009)).

AP endonuclease 1

APE1 is a 318 aa-long protein with three regions (reviewed in (Tell *et al.*, 2010)). The 35 N-terminal aa of APE1 contains a NLS and is involved in protein-protein interactions. Truncation of the NLS containing region of APE1 causes translocation to mitochondria (Chattopadhyay *et al.*, 2006). Many proteins that have been reported to interact with APE1are known factors involved in BER (reviewed in (Tell *et al.*, 2010)). The aa 35-127 region of APE1, known as the redox region, is capable of altering the DNA binding of several transcription factors through reduction of Cys residues in proteins, such as AP1, NF-κB, and p53 (Xanthoudakis *et al.*, 1992; Gaiddon *et al.*, 1999; Nishi *et al.*, 2002). The aa 161-318 region of APE1 harbors its AP endonucleases activity that nicks AP sites and produces SSBs with 3' hydroxyl and 5' dRP termini (Mol *et al.*, 2000). In addition to AP site cleavage, APE1 converts 3'-dRP termini, *e.g.* produced by bifunctional DNA glycosylases through β-elimination. APE1 also converts 3' phosphoglycolate termini, produced during ROS mediated SSBs, to 3'-hydroxyl termini (Pascucci *et al.*, 2002; Marenstein *et al.*, 2003; Parsons *et al.*, 2004). Mammalian APE1 has 3' phosphatase activity but *in vitro* activity analysis strongly suggests that PNKP is the main 3' phosphatase in mammalian cells (Wiederhold *et al.*, 2004).

Polynucleotide kinase/phosphatase

PNKP is a 521 aa long protein. Its catalytic activity resides in a C-terminally fused 3' DNA phosphatase and 5' kinase domain (Breslin *et al.*, 2009). Mammalian PNKP phosphorylates 5'-hydroxyl termini of preferentially nicked, gapped dsDNA and DSBs with 3' overhangs, and has phosphatase activity on a variety of 3' phosphate termini on

both dsDNA and ssDNA (Karimi-Busheri *et al.*, 1997; Bernstein *et al.*, 2009). 3'-phosphate ends are a product of δ-elimination by the NEIL family of bifunctional DNA glycosylases, making an APE1 independent BER pathway through PNKP plausible (figure 1. Step II) (Wiederhold *et al.*, 2004).

Gap filling/5'terminus tailoring

The second major branching point in BER and first in SSBR occurs during gap filling. DNA polymerases synthesize either one or several nucleotides, coined short patch (SP) and long patch (LP) BER (or SSBR) respectively (figure 1. Step III) (Dianov *et al.*, 1992; Frosina *et al.*, 1996). During SP BER, the 5'-dRP termini produced by APE1 cleavage of AP-sites must be removed before ligation may occur. The major 5'-dRP lyase activity in mammalian cells corresponds to POLβ and is, combined with POLβ's DNA synthesis activity, central to mammalian SP BER capacity (Sobol *et al.*, 2000; Allinson *et al.*, 2001; Podlutsky *et al.*, 2001b). However, several other DNA polymerases have both DNA synthesis and dRP lyase activity, and have been proposed to be involved in BER: POLγ, POLθ, POLι, and POLλ (Longley *et al.*, 1998; Bebenek *et al.*, 2001; Braithwaite *et al.*, 2005; Prasad *et al.*, 2009). POLβ is also central to LP BER and has been shown to initiate gap filling by insertion of one or two nucleotide(s), followed by a switch to POLδ or POLε (Dianov *et al.*, 1999; Podlutsky *et al.*, 2001a; Akbari *et al.*, 2009). The mechanisms that underlie the balance between LP and SP BER are elusive and probably complex because very disparate molecular mechanisms have been shown to influence it: *e.g.* cell cycle regulation, stress responses, lesion type, and the expression levels of involved proteins (Fortini *et al.*, 1999; Dogliotti *et al.*, 2001; Akbari *et al.*, 2009; Sukhanova *et al.*, 2010). Furthermore, presence of 5'-termini that cannot be processed into 5'-phosphate would require strand displacement (*i.e.* LP BER) (Dogliotti *et al.*, 2001). Strand displacement in LP BER involves DNA replication factors such as PCNA and Flap endonuclease 1 (FEN1) (reviewed in (Sung *et al.*, 2006)).

DNA Polymerase β

The smallest of all known eukaryotic polymerases, POLβ (39 kDa), is a member of the X family of polymerases and has two specialized domains. The dRP activity is located in an N-terminal domain of 8 kDa while the remaining C-terminal 31 kDa domain contains both nucleotidyl activity and dsDNA binding (template/primer) capacity (Prasad *et al.*, 1998). POLβ misincorporates nucleotides at rates of more than 10^{-4} errors per synthesized nucleotide, and since lesions repaired by BER exceed 10^4 lesions per cell per day proofreading is vital to avoid genomic degradation (reviewed in (Lindahl, 1993; Yamtich *et al.*, 2010)). Unlike replicative polymerases, POLβ does not have 3'-5' exonuclease activity (Beard *et al.*, 2006). However, proofreading during BER has been proposed to be carried out by ligase discrimination and 3'-5' exonuclease activities of AP endonucleases towards mispaired 3' residues (Bhagwat *et al.*, 1999; Chou *et al.*, 2002; Burkovics *et al.*, 2006).

POLβ deficiency in mice is embryonically lethal, although cells from the embryo are viable in culture, indicating that POLβ is vital to fetal development but not in cellular viability (Gu *et al.*, 1994). *Polβ* knockout cell lines are highly sensitive to methylating agents. Interestingly, this sensitivity can be rescued by transfection of a truncated POLβ without DNA synthesis capacity but with dRP lyase activity intact (Ochs *et al.*, 1999). POLβ has been shown to interact with many factors involved in DNA repair, replication, and cell cycle regulation (reviewed in(Almeida *et al.*, 2007)).

Flap endonuclease 1

FEN1 is a member of the XPG/RAD2 endonuclease family with both 5' exonuclease and flap endonuclease activities (Harrington *et al.*, 1994; Murante *et al.*, 1994).

The 5' exonuclease activity converts DNA nicks to gaps, thus permitting DNA polymerases to initiate DNA synthesis (Liu *et al.*, 2005). FEN1 is predominantly a flap endonuclease and cleaves 5'-flaps in their ssDNA/dsDNA junctions resulting in ligatable nicked dsDNA products. FEN1 is best known for its involvement in Okazaki fragment maturation during DNA replication and LP BER, but is also involved in

metabolic pathways, maintenance of telomeres, and apoptosis (reviewed in (Zheng *et al.*, 2011)).

The coordination of FEN1 activities in diverse pathways is probably influenced by its many protein interactions. There were 34 FEN1-interacting proteins in the STRING[4] database as of 2010, including the scaffolding factors PCNA and 9-1-1 (RAD9-RAD1-HUS1) (Zheng *et al.*, 2011). The 9-1-1 complex is believed to work as a DNA damage sensor, and like PCNA it stimulates the flap endonuclease activity of FEN1 (Wang *et al.*, 2004). Although still elusive, the regulation and coordination of FEN1 in LP BER involve interactions with POLβ, APE1, LIG1, PCNA and PARP1 (Gary *et al.*, 1999; Dianova *et al.*, 2001; Lavrik *et al.*, 2001; Pascal *et al.*, 2004). FEN1 deficiency causes DNA replication defects and is embryonically lethal in mice (Larsen *et al.*, 2003; Zheng *et al.*, 2007).

Proliferating cell nuclear antigen

PCNA is a homotrimeric donut-shaped factor best known for its role as a DNA sliding clamp that provides stability for the replication machinery (Moldovan *et al.*, 2007). Nevertheless, PCNA also interacts with a long list of factors involved in DNA repair, chromatin remodelling, cell cycle regulation, and cell survival, and has been classified as a central scaffolding factor involved in maintaining the integrity of proliferating cells (Maga *et al.*, 2003). PCNA has recently been observed to regulate survival of fully differentiated neutrophiles through sequestration of procaspases in the cytosol, further broadening PCNA's roles as a platform protein and its involvement in cellular processes (Witko-Sarsat *et al.*, 2010).

PCNA expression levels are modulated throughout cell cycle and peak in S-phase during which the numerous proteins involved in replication assemble in clusters within the nucleus. These replication clusters can be observed in live cells by conjugating fluorescent proteins to the involved factors and visualized by confocal microscopy. In

[4] Search Tool for the Retrieval of Interacting Genes/Proteins
http://string-db.org

early S-phase, the foci are small and scattered in the nucleus. In late S-phase, they assemble around nucleoli and close to the nuclear membrane (Leonhardt *et al.*, 2000). Proteins involved in BER that interact with PCNA include: glycosylases (UNG2, MYH, NTH and MPG), DNA polymerases (POLβ, POLδ, and POLε), FEN1, LIG1, PARP1, and XRCC1 (Eissenberg *et al.*, 1997; Levin *et al.*, 1997; Loor *et al.*, 1997; Otterlei *et al.*, 1999; Zhang *et al.*, 1999; Parker *et al.*, 2001; Kedar *et al.*, 2002; Frouin *et al.*, 2003; Fan *et al.*, 2004; Oyama *et al.*, 2004; Xia *et al.*, 2005).

The interaction between PCNA and XRCC1 and the associated clustering of XRCC1 to the replication machinery, indicates involvement of BER/SSBR factors in replication (Fan *et al.*, 2004). BER/SSBR contribution to replication is probably dual, because the consequences of lesions differ if they are either encountered (pre-replicative) or produced (post-replicative) by the replication machinery. Pre-replicative lesions with toxic potential repaired by BER/SSBR would include mutagenic lesions such as C deaminated to U (transition mutations), and lesions that cause replication block (*e.g.* thymine glycol and 3-meA) or replication collapse (SSB) (Caldecott, 2003; Akbari *et al.*, 2010). Post-replicative lesions assessed by BER/SSBR would include base lesions such as misincorporated U and A opposite 8oxoG (Otterlei *et al.*, 1999; Boldogh *et al.*, 2001).

Ligation

The final step of BER/SSBR requires fusion of the nicked DNA backbone. DNA ligases catalyze phosphodiester bond formation through a three-step reaction mechanism that requires a high-energy cofactor (ATP in eukaryotes) and adjacent 3'-hydroxyl and 5'-phosphate termini. To date, three DNA ligase genes have been identified in mammals: *Lig1*, *Lig3,* and *Lig4* (Barnes *et al.*, 1990; Chen *et al.*, 1995; Wei *et al.*, 1995). While homologs of *Lig1* and *Lig4* seem to be present in all eukaryotic cells, *Lig3* has only been found in vertebrates and encodes three distinct DNA ligases, *i.e.* the ubiquitously expressed nuclear and mitochondrial translational initiation variants of DNA ligase 3α (LIG3α) and a germ cell-specific alternative splice variant DNA ligase 3β (LIG3β) (reviewed in (Ellenberger *et al.*, 2008)). LIG4 does not seem to participate in BER/SSBR, because it only faintly accumulates in nuclear regions irradiated with UVA

(Mortusewicz *et al.*, 2006), and little is known about LIG3β (reviewed in (Ellenberger *et al.*, 2008)). The respective interactions between the scaffolding factors XRCC1 and PCNA with LIG3α and LIG1 indicate a distinct involvement in LP and SP BER sub pathways. While biochemical experiments confirms lack of participation of LIG3α in LP, LIG1 is both SP and LP proficient (Cappelli *et al.*, 1997; Sleeth *et al.*, 2004).

Ligase 3

Mitochondrial LIG3α is a 1009 aa long polypeptide with a N-terminal mitochondrial localization signal (MLS), followed by a SSB-binding zinc finger, a central DNA binding domain, a catalytic domain, and a C-terminal BRCT domain. Nuclear LIG3α lacks the MLS (reviewed in (Ellenberger *et al.*, 2008)).

There are strong indication of LIG3α involvement in BER/SSBR: the XRCC1/ LIG3α complex resists treatment with high salt concentrations, LIG3α recruitment to DNA damage foci depends on XRCC1, XRCC1 and LIG3α deficiencies are both embryonically lethal in mice with similar phenotypes, and in addition to XRCC1 other factors involved in BER/SSBR interact with LIG3α (*e.g.* PARP1 and TDP1) (Caldecott *et al.*, 1995; Tebbs *et al.*, 1999; Leppard *et al.*, 2003; El-Khamisy *et al.*, 2005; Mortusewicz *et al.*, 2006; Puebla-Osorio *et al.*, 2006). Assessing the *in vivo* contribution of LIG3α to BER/SSBR has been difficult, since *Lig3* deficient cells have long proven unviable (Puebla-Osorio *et al.*, 2006). However, the viability of *Lig3*$^{-/-}$ murine embryonic stem cells (mES) was recently rescued by expressing mitochondrial LIG3α, but not nuclear LIG3α (Simsek *et al.*, 2011). The viability depended exclusively on mitochondrial ligase activity since mitochondrial expression of a zinc-finger domain and BRCT truncated LIG3α, LIG1, or ligases from either *Chlorella* virus or *E. Coli* could rescue *Lig3*$^{-/-}$ mES. Furthermore, rescued *Lig3*$^{-/-}$ mES did not show any hypersensitivity to methylation, ROS, UV, IR or PAR inhibition. This apparent lack of requirement of nuclear LIG3α was also observed in *Lig3* deficient mice astrocytes successfully harvested from mice by tissue specific disruption of *Lig3* (Gao *et al.*, 2011). While the *Lig3*$^{-/-}$ astrocytes showed severe mitochondrial dysfunction, causing neuronal defects in the mice, the repair capacities of methylation, ROS or IR induced lesions were not reduced compared to wild-type. However, repair capacity was reduced

when knocking down LIG1 expression, and even stronger when knocking down both LIG1 and LIG3α. These results indicate that the roles of the nuclear XRCC1/ LIG3α complex are elusive, and potentially redundant to LIG1 or diverging between quiescent and proliferating cells.

Aims of the study

The many XRCC1 protein interactions that have been reported, including the versatile scaffolding factor PCNA, have long indicated assembly of large BER proficient multiprotein complexes potentially involved in disparate DNA processing pathways. The overall aim of this thesis is to describe the composition, regulation and the involvements of such complexes by both *in vivo* confocal imaging techniques and *in vitro* biochemical methods that attempt to preserve the interactions within multiprotein complexes.

We have aimed to address the following questions:

A. Is XRCC1-mediated BER coupled to replication?

B. Does DNA damage context influence XRCC1 scaffolding properties and complex properties?

C. How do the different regions of XRCC1 participate in XRCC1 scaffolding properties?

D. Is XRCC1 recruitment to DNA damage regulated by kinase signalling?

Manuscript/publication summaries

Paper 1: DNA repair (Amst.) 2010

Direct interaction between XRCC1 and UNG2 facilitates rapid repair of uracil in DNA by XRCC1 complexes

Akbari, M., Solvang-Garten, K.*, Hanssen-Bauer, A.*, Lieske, N. V., Pettersen, H. S., Pettersen, G. K., Wilson, D. M. 3rd., Krokan, H. E., Otterlei, M.
*These authors contributed equally to this work

The previous observations that both UNG2 and XRCC1 are recruited to the replication machinery through interaction with PCNA (Otterlei *et al.*, 1999; Fan *et al.*, 2004), the reported interactions between multiple DNA glycosylases and XRCC1 (Campalans *et al.*, 2005), and an observed UDG activity of recombinant XRCC1 protein purified from *E. Coli* prompted us to examine whether there is a direct interaction between UNG2 and XRCC1 at replication foci. Confocal microscopy of fluorescently-tagged UNG2, XRCC1, and PCNA expressed in HeLa cells clearly showed, overlapping subnuclear localization to replication foci in S-phase. A direct interaction between UNG2 and XRCC1 was suggested by the results of the proximity ligation assay (PLA). PLA uses antibodies with conjugated DNA strands as proximity probes combined with rolling circle amplification and detection by fluorescent DNA probes. The functional distance between two targets producing a positive signal is close to FRET distance and thus a strong indication of protein-protein interaction (reviewed in (Weibrecht *et al.*, 2010)).

Chinese hamster XRCC1 (cXRCC1) deletion constructs immunoprecipitated (IPed) from EM9 CHO (*Xrcc1*$^{-/-}$), washed in high salt, and incubated with the catalytic domain of human UNG2 (Δ93 UNG2) demonstrated that Δ93 UNG2 interacts with the XRCC1 region encompassing the NLS (aa 183-315). Because both XRCC1 and UNG2 interact with PCNA, direct cXRCC1 / Δ93 UNG2 interaction was confirmed by Far Western analysis. The presence of UNG2 in XRCC1 complexes was further confirmed by abrogation of U:A and U:G repair capacity of XRCC1-EYFP immunoprecipitates when

treated with the UNG inhibitor Ugi or antibodies targeted against the catalytic domain of UNG.

In addition to the observed co-localization of UNG2, XRCC1, and PCNA in replication foci, several XRCC1 specific foci without UNG2 or PCNA were detected in both S- and non-S-phase cells, suggesting the presence of discrete XRCC1 complexes. Immunoprecipitation (IP) of XRCC1 or UNG2 from S-phase and G1/S-phase enriched and mildly formaldehyde crosslinked HeLa cells confirmed the presence of distinct complexes throughout cell cycle. The crosslinked XRCC1 immunoprecipitates contained high molecular weight complexes (HMW) (>200 kDa) with *e.g.* POLδ. In agreement with our confocal results, the yield of HMW XRCC1 complexes was higher in S-phase lysates and contained more UNG2 and PCNA compared with G1/S-phase lysates. The presence of UNG2 and XRCC1 in a common complex was also detected by IP of UNG2 and XRCC1. However, as the confocal results suggested, we found distinct differences in the protein composition of XRCC1 and UNG2 immunoprecipitates. LIG1 only co-IPed with UNG2, while LIG3, POLβ, and PNKP co-IPed more readily with XRCC1-EYFP. Similar results were found when immunoprecipitating XRCC1 or UNG2 from chromatin-enriched lysates of freely cycling cells.

To determine differences in repair proficiency between S-phase XRCC1 and UNG2 complexes we tested their BER activity on U:A, U:G, and AP site substrates. XRCC1 complexes performed complete BER on all substrates. In contrast, UNG2-complexes were unable to efficiently generate the final ligated product despite the presence of LIG1. The inefficient ligation was not affected by addition of APE1, but was rescued by addition of APE1 and T4 Ligase. Prolonged repair time and addition of merely APE1 only partially rescued the production of the final ligated product, indicating that the UNG2 complex is inefficient in both SSB end-trimming and ligation. In summary western analysis and BER activity analysis indicated that XRCC1 and UNG2 in S-phase are present in common complexes as well as in functionally different complexes.

The interaction between XRCC1 and UNG2 further prompted us to assess whether XRCC1 influences UNG2 specific U repair. Cell extracts from EM9 (*Xrcc1*$^{-/-}$), its

parental wild-type cell line AA8 (*Xrcc1*$^{+/+}$), and EM9 expressing exogenous cXRCC1 were tested for their ability to repair U:A and AP site containing substrates and excise U from DNA. XRCC1-deficient cell extracts were considerably slower in their U:A repair. It is unlikely that the reduced U:A repair was due to lack of stabilizing effects of XRCC1 on POLβ and LIG3 or reduced uracil-DNA-glycosylase activity, because the XRCC1-deficient cell extracts did not present reduced AP site repair nor Uracil-DNA glycosylase activity. Our results thus indicate that XRCC1 plays a specific role in the coordination of repair of U:A.

In summary this paper shows a direct interaction between the NLS region of XRCC1 and the catalytic domain of UNG2 in replication associated-complexes. Furthermore, we have identified functional differences between XRCC1- and UNG2- associated S-phase complexes. Based on these and earlier observations we present a model suggesting pre-replicative BER targeting removal of mutagenic U:G mismatches, in addition to post-replicative excision of U:A misincorporations that are later repaired by XRCC1 BER complexes.

Paper 2: Environmental and Molecular Mutagenesis 2011

XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage.

Hanssen-Bauer, A.*, Solvang-Garten, K.*, Sundheim, O., Pena-Diaz, J., Andersen, S., Slupphaug, G., Krokan, H. E., Wilson, D. M.3rd., Akbari, M., Otterlei, M.
* These authors contributed equally to this work

XRCC1 is considered a central BER/SSBR scaffolding factor because of its many interactions with factors involved in BER/SSBR and the phenotypes of XRCC1-deficient cells. The extensive overlap of interacting regions in XRCC1 has further led to a notion of XRCC1 organizing BER through sequential interaction with one or several enzymatic factors. However, both *in vitro* and *in vivo* data strongly suggest that XRCC1 forms multimers, either through direct interactions between its BRCT domains or indirectly through the factors that bind to the BRCT domains (Fan *et al.*, 2004; Beernink *et al.*, 2005; Levy *et al.*, 2006; Cuneo *et al.*, 2011). Multimeric XRCC1

complexes would be able to incorporate factors with overlapping XRCC1 interactions into the same multiprotein complex. Furthermore, XRCC1 can interact with the scaffolding factor PCNA. Because "*[t]here is hardly any protein known to date that rivals PCNA in its capacity to associate with so many different and alternative cofactors[.]*" (Moldovan *et al.*, 2007), it seems plausible that XRCC1 takes part in multimeric protein complexes capable of assembling many repair factors. Our observation of large XRCC1 complexes containing several BER factors, and the association of XRCC1 to the replication machinery during S-phase (paper 1) is in agreement with this hypothesis.

In this paper we demonstrated that lysates from freely cycling cells contain XRCC1-associated protein complexes with molecular weights ranging from 150-1500 kDa. BER assay analysis indicated that complexes ranging between 350-700 kDa had the highest capacity to initiate BER of AP sites, and contained detectable amounts of PNKP, POLβ, POLδ, LIG3, and PCNA. APE1 was not detected, but its presence is highly probable because IPed complexes were able to process AP site substrates. Furthermore, our previous results (paper 1) showed that APE1 only was detected when its interaction with XRCC1 complexes was fixed by formaldehyde crosslinking. UNG2 was identified in immunoprecipitates with molecular masses either above 670 kDa or below 440 kDa. Presence of UNG2 in high molecular mass complexes is in agreement with our observations in paper 1, where UNG2 was shown to be associated to large XRCC1 containing complexes in S-phase. The presence of UNG2 in low molecular mass might indicate that complexes are broken down and/or new interactions are formed during the *in vitro* handling. However, the results confirm the observation that XRCC1 takes part in large multiprotein complexes containing factors capable of contributing to all BER steps.

The presence of both POLβ and POLδ suggests that the XRCC1 complexes are both SP and LP BER proficient. We therefore assessed the POL's respective contribution to insertion of the first or the following nucleotides by using IPed XRCC1-EYFP complexes from freely cycling cells and AP site containing substrate. Inhibition of POLδ reduced first nucleotide incorporation by only 27% within the first 15 min and

57% after 45 min. Incorporation of the second and the following nucleotides increased with time in absence of POLδ inhibitor, but remained undetectable when POLδ was inhibited. Neutralizing antibodies targeting POLβ activity reduced overall BER activity. Together these results indicate that nucleotide incorporation after the first nucleotide was exclusively performed by POLδ, and confirms earlier observations that POLβ is central for the insertion of the first nucleotide (Podlutsky *et al.*, 2001a). LP BER was further analyzed by challenging the IPed complexes with substrates containing a synthetic analog of AP sites, 3-hydroxy-2-hydroxymethyltetrahydrofuran (THF), which cannot be processed by the lyase activity of POLβ but requires strand displacement and flap endonuclease activity. When detecting first nucleotide insertion of XRCC1 complexes on AP site substrate, all detected repair products were ligated within 15 min, while a fraction of the gap filled THF substrate remained unligated. The incomplete THF substrate repair indicates that LP BER is less efficient than SP BER. Furthermore, AP site repair seems to mainly be repaired by SP BER as nucleotide insertion after the first nucleotide was not detected. However, nucleotide insertion of the second and following nucleotides was observed in THF substrate repair, confirming both that THF sites require LP BER and that XRCC1-EYFP complexes are LP BER proficient.

By confocal imaging and fluorescent reporter proteins we show that PNKP and POLβ co-localize with XRCC1 both in and outside of replication foci. Neither PCNA nor UNG2 are found in foci outside of S-phase, and thus do not seem to participate in the repair of endogenously produced SSB and base lesions. Lan *et al.* have reported that PCNA accumulates in UVA (365 nm) mIF (Lan *et al.*, 2004). However, the laser dose or the type of DNA damage induced in these experiments was not indicated. Because longer wavelength UVA mainly induces SSB and ROS, we used a near-UVA (405 nm) laser to assess the requirement of POLβ, PNKP and PCNA to mIF.

First, we tested the requirement for XRCC1 in the recruitment of POLβ, PNKP, and PCNA by near-UVA microirradiation of EM9 and AA8 CHO expressing fluorescently-tagged proteins. POLβ was found to be completely dependent of XRCC1 (human or hamster), while the recruitment of PNKP and PCNA required lower doses with XRCC1 present. Over-expression of human XRCC1 also lowered the required dose for the

treated with the UNG inhibitor Ugi or antibodies targeted against the catalytic domain of UNG.

In addition to the observed co-localization of UNG2, XRCC1, and PCNA in replication foci, several XRCC1 specific foci without UNG2 or PCNA were detected in both S- and non-S-phase cells, suggesting the presence of discrete XRCC1 complexes. Immunoprecipitation (IP) of XRCC1 or UNG2 from S-phase and G1/S-phase enriched and mildly formaldehyde crosslinked HeLa cells confirmed the presence of distinct complexes throughout cell cycle. The crosslinked XRCC1 immunoprecipitates contained high molecular weight complexes (HMW) (>200 kDa) with *e.g.* POLδ. In agreement with our confocal results, the yield of HMW XRCC1 complexes was higher in S-phase lysates and contained more UNG2 and PCNA compared with G1/S-phase lysates. The presence of UNG2 and XRCC1 in a common complex was also detected by IP of UNG2 and XRCC1. However, as the confocal results suggested, we found distinct differences in the protein composition of XRCC1 and UNG2 immunoprecipitates. LIG1 only co-IPed with UNG2, while LIG3, POLβ, and PNKP co-IPed more readily with XRCC1-EYFP. Similar results were found when immunoprecipitating XRCC1 or UNG2 from chromatin-enriched lysates of freely cycling cells.

To determine differences in repair proficiency between S-phase XRCC1 and UNG2 complexes we tested their BER activity on U:A, U:G, and AP site substrates. XRCC1 complexes performed complete BER on all substrates. In contrast, UNG2-complexes were unable to efficiently generate the final ligated product despite the presence of LIG1. The inefficient ligation was not affected by addition of APE1, but was rescued by addition of APE1 and T4 Ligase. Prolonged repair time and addition of merely T4 Ligase only partially rescued the production of the final ligated product, indicating that the UNG2 complex is inefficient in both SSB end-trimming and ligation. In summary western analysis and BER activity analysis indicated that XRCC1 and UNG2 in S-phase are present in common complexes as well as in functionally different complexes.

The interaction between XRCC1 and UNG2 further prompted us to assess whether XRCC1 influences UNG2 specific U repair. Cell extracts from EM9 (*Xrcc1*$^{-/-}$), its

and FEN1 accumulation followed the accumulation of PARP1 in 4-AN and PJ34 treated cells.

To summize this paper confirmed the presence of XRCC1 in mulitiprotein SP/LP BER proficient complexes of variable composition. Our results also showed that PNKP and POLβ co-localize with XRCC1 in replication foci and in both constitutively present and induced foci throughout the cell cycle. Furthermore, we showed that recruitment of PNKP and POLβ by low dose UVA induced damage requires XRCC1. We also confirmed that PCNA does not co-localize with constitutively present XRCC1 complexes and demonstrated that PCNA can be recruited together with FEN1 to sites of DNA damage and XRCC1 foci, depending on the extent and type of damage induced. Our results confirmed the involvement of POLβ in first nucleotide insertion during gap filling, and indicated that it does not participate with insertion of the following nucleotides in competition with POLδ. We also demonstrated that PARylation is not crucial for recruitment of BER factors, and our results suggest that XRCC1 complex accumulation at DNA lesions is independent of PARP1 interaction. Based on these results, we proposed that XRCC1 is found in at least three multiprotein complexes: associated to replication, in a SP BER proficient "core complex" induced by low levels of DNA degradation, and in a SP/LP BER proficient complex induced by high levels of damage.

Paper 3: DNA repair (Amst.) (accepted January 2012)

The region of XRCC1 from residues 310 to 436, which harbours the three most common nonsynonymous polymorphic variants, is essential for the scaffolding function of XRCC1

Hanssen-Bauer A.*, Solvang-Garten K.*, Gilljam K.M., Thorseth K., Wilson D.M. 3rd, Akbari M., Otterlei M.

*These authors contributed equally to this work

XRCC1 contributes to BER as a scaffolding protein through its many protein interactions and its capacity to be recruited to sites of DNA lesions and association with the cells replication machinery (paper 1 and 2). Proteins have been shown to interact

with regions of XRCC1 throughout its whole peptide sequence, and the associated proteins show variable dependency on XRCC1 for recruitment to lesions (paper 2). In this paper we examined the contribution of three different XRCC1 regions to its scaffolding properties, and attempted to assess the impact of the most common polymorphic variants of XRCC1.

To determine the XRCC1 region necessary for its recruitment to lesions we performed near-UVA microirradiation in XRCC1-deficient cells expressing deletion constructs and full length XRCC1. We used laser doses sufficient for recruitment of PNKP and POLβ, but not PCNA and below the doses necessary for accumulation of the DSB marker γH2AX (paper 2). Deletion constructs ranging between aa 166-436 (MD) and aa 311-633 (BLB) were recruited similarly to full length XRCC1, a rapid accumulation reaching a maximum intensity after approximately 100 s. However, while the MD construct and full length XRCC1 foci reached a ~2-fold increase in signal compared to the background, the BLB mutant foci only reached a ~1.4-fold increase. These results suggest that the overlapping region between MD and BLB (aa 311-436; mainly BRCT1) is required for XRCC1 recruitment to lesions, while the MD specific region (aa 166-311; between N-terminal domain and BRCT1) is particularly important for the localization properties of XRCC1. The deletion construct ranging from aa 1-310 (XNTD) did not accumulate into foci, even when the irradiation dose was increased 100-fold.

XRCC1 co-localizes with PCNA in replication foci independent of DNA damage (Fan et al 2004). Although only the MD and XNTD deletion constructs contained the PCNA interacting region of XRCC1 (aa 152-315), confocal imaging revealed that BLB also co-localizes with PCNA in replication foci. The lack of recruitment of XNTD indicates that the PCNA binding domain is not sufficient and that the BRCT1 region is required for localization to replication foci. The presence of BLB implies that XRCC1 can be recruited via other proteins.

To further assess the contribution of the different XRCC1 regions to BER, we performed Comet assay on *Xrcc1*$^{-/-}$ EM9 CHO cells expressing XRCC1 deletion

constructs and treated the cells with either methyl methanesulfonate (MMS) or H_2O_2. While the XNTD and BLB constructs did not alter the accumulated fragmentation compared to the negative control, the MD construct partially complemented full length XRCC1 expression after MMS treatment. These results indicate that one or several of the specific interactions within the MD region of XRCC1 is critical for efficient repair of MMS-induced damage, but dispensable for repair of ROS-induced damage.

The three most prevalent variants of XRCC1, Arg194Trp, Arg280His, and Arg399Gln, are all located within the MD construct. Their contributions to cancer risk have been assessed in numerous epidemiological studies and, although with diverging results, seem to be associated with cancer in combination with life style factors. We could not detect significant differences in the cellular localization or capacity to recruit PNKP or POLβ to mIFs when comparing the XRCC1 variants with the conservative XRCC1. However, we did detect slightly reduced abilities to accumulate or remain in mIFs and slight differences in the repair profiles of MMS and H_2O_2-induced damage as measured by alkaline Comet assay. Small variations in recruitment to exogenously-induced DNA damage could support an association between these polymorphism and increased cancer risk in relation to life-style factors such as smoking.

This paper shows that the BRCT1 domain (aa 315-403) is crucial for recruitment of XRCC1 to lesions, while the region spanning between the N-terminal domain and BRCT1 (aa 166-311) influences its recruitment properties. The aa 116-403 region plays a specific role in repair of MMS induced damage and is required for efficient recruitment of PCNA to lesions, probably through its PCNA interaction region. However, the PCNA interacting region is not required for XRCC1 localization to replication foci. Finally, the three most prevalent polymorphisms of XRCC1 have slightly reduced recruitment capacities to lesions and slightly reduced BER capacities.

Pages 50-52 have been removed from the electronic version

Discussion

The original observations made by Thompson *et al*. that CHO cells defective in XRCC1 present phenotypes indicating involvement in repair of base lesions and strand breaks (i.e. hypersensitivity to methylating agents, ROS and IR) have later been confirmed by the accumulation of reported XRCC1 interactions with DNA glycosylases and other enzymes capable of processing BER intermediates ((Thompson *et al.*, 1990), reviewed in (Almeida *et al.*, 2007)). This thesis centers on the notion of XRCC1 as a central BER scaffolding factor. However, proteins and their involvement in complex pathways have likely evolved from already existing factors and pathways. In accordance with this, involvement of XRCC1 in other repair pathways, such as NER and NHEJ, has been reported (Audebert *et al.*, 2004; Moser *et al.*, 2007). Furthermore, XRCC1-deficient cancer cell lines proliferate readily and are capable of repairing their genome after insults causing base lesions and strand breaks. Thus, it should be noted that there is extensive crosstalk and redundancy among DNA repair pathways.

XRCC1 complexes are large multimers

The observation that BER can be reproduced *in vitro* with as few as four enzymes and the existence of more than twenty BER factors capable of processing a broad selection of lesions and repair intermediates through different sub-pathways of BER has lead to a notion of BER as a succession of enzymatic events. In this regard, XRCC1 mainly contributes as a co-factor or temporary docking platform because it only interacts with a selection of the BER factors (e.g. roughly half of the DNA glycosylases, only one ligase and polymerase). Furthermore, only the interactions with POLβ and LIG3 are considered strong and contribute to proteolytic stability, and the remaining interactions partners show extensive overlap scattered all along the XRCC1 peptide sequence. However, the dimeric interactions between XRCC1 BRCT domains and heterotetrameric assembly of XRCC1 with either PARP1 or LIG3 observed *in vitro*, and FRET results have long indicated the possibility of assembly of XRCC1 complexes able to contain many factors (Fan *et al.*, 2004; Beernink *et al.*, 2005; Levy *et al.*, 2006; Cuneo *et al.*, 2011). The assembly of large multiprotein complexes is plausible because many of the XRCC1 interacting proteins have been shown to interact among themselves (reviewed in (Almeida *et al.*, 2007)) and XRCC1 interacts with another well known

scaffolding factor, PCNA. In 2004 our group isolated a large BER proficient multiprotein complex by pulling down UNG2, containing PCNA, XRCC1, and factors that overlap in their interaction with XRCC1 (Akbari *et al.*, 2004). Similar results were obtained by Heale *et al*. and Parlanti *et al,.* who also observed factors in the complex that are not known to interact directly with XRCC1 (*e.g.* POLδ, POLε, and FEN1) (Heale *et al.*, 2006; Parlanti *et al.*, 2007). Our results in paper 1 confirmed the presence of multiprotein XRCC1-EYFP-associated complexes containing BER factors with overlapping XRCC1 interactions (PCNA and PARP1 vs. UNG2 vs. APE1) as well as factors with no known interactions with XRCC1 (POLδ, FEN1, and LIG1) and endogenous XRCC1. The presence of such XRCC1 complexes was further confirmed by gel fractionation of immunoprecipitated XRCC1 complexes in paper 2. *In vitro* studies of the interaction between BER enzymes and their DNA substrates indicate that the enzymes stays bound to their product, preventing toxic and mutagenic effects of repair intermediates before handing it over to the following enzyme (Prasad *et al.*, 2010). Multiprotein BER complexes could contribute to tethering factors to the lesions and repair intermediates and thus potentially secure progression through the pathway, increase the rate of repair, and provide a platform that can handle irregularities through different paths or possibly several lesions in parallel.

Variable complex compositions

The involvement of replication factors such as POLδ, POLε, FEN1, and PCNA in LP BER and the deleterious effects of replication past SSB and certain base lesions have lead to the hypothesis of replication-coupled BER / SSBR (reviewed in (Caldecott, 2001)). The UNG2-associated complexes described by our group in 2004 contained both XRCC1 and PCNA while having a predominant SP BER activity (Akbari *et al.*, 2004). The presence of XRCC1 and UNG2 in the replication complex was confirmed by Parlanti *et al.* (Parlanti *et al.*, 2007). In paper 1 we demonstrated that the composition of XRCC1-associated complexes varies throughout cell cycle. Furthermore, we showed that although UNG2 and XRCC1 interact directly, UNG2 and XRCC1 immunoprecipitates from S-phase cells contain both common and separate factors and present different BER proficiencies. Confocal imagery (papers 1 and 2) indicated that XRCC1, PNKP and POlβ are found both within and outside of replication foci, while

UNG2 is only found in association with the replication machinery. This confirms the presence of XRCC1 mediated BER / SSBR associated to replication and points to the presence of distinct BER complexes. This was further supported by the gel fractionation results in paper 2, which demonstrated the presence of BER proficient XRCC1 complexes of variable size and composition. Notably, we found that the dose of near-UVA laser used to induce DNA lesions influenced the repertoire of DNA repair proteins recruited. Low doses resulted in recruitment of factors that are also observed in foci independent of exogenously induced DNA damage, *e.g.* PNKP, and POLβ, while high doses in addition triggered recruitment of PCNA and FEN1. In sum our observations suggest the presence of a "XRCC1 core complex" that assembles at sites of endogenous or low levels of exogenous damage and is extended to include proteins involved in LP BER as well as other repair pathways when levels of damage reach a certain threshold. This XRCC1 core complex is found associated to replication where it interacts with DNA glycosylases such as UNG2. Based on western analysis of immunoprecipitated complexes and near-UVA laser mIF, we propose that the XRCC1 core complex includes PNKP, POLβ, and LIG3. Western analysis of formaldehyde crosslinked complexes in paper 1 and BER activity of low mass complexes in paper 2 further suggest that APE1 is also a member of the XRCC1 core complex. Our results do not exclude the presence of other polymerases, end-trimming enzymes, or glycosylases that were not included in our analysis. However, caution should be exercised when determining complex composition and activity. The presence of UNG2 in XRCC1-associated complexes below 440 kDa in paper 2 is not in accordance with its observed presence in large replication-associated complexes in paper 1, and is most likely an artifact occurring during *in vitro* handling. Furthermore, Akbari *et al.* recently demonstrated that BER activity of nuclear extracts and UNG2 is influenced by the volume of the lysate extraction buffer (Akbari *et al.*, 2011).

Complex regulation and recruitment

The variable composition and rapid recruitment of XRCC1 multiprotein complexes observed in paper 1 and 2 suggest regulation of protein interactions through PTM. An overview of possible PTMs that influence recruitment and complex composition of XRCC1 multiprotein complexes is beyond the scope of this thesis since it probably involves interactions between proteins that do not include XRCC1. However, it should be noted that these potentially are just as important as direct interactions with XRCC1.

PARP1 affinity for and activation by SSB and its reported interaction with the BRCT1 domain of XRCC1 makes it a plausible candidate as initiator of XRCC1 complex recruitment to SSB (Masson *et al.*, 1998). El-Khamisy *et al*. reported immunohistochemistry results that demonstrated abrogation of XRCC1 foci formation by inhibition of PARylation, and foci reduction by point mutation within a putative PAR binding motif in BRCT1, suggesting that XRCC1 recruitment to SSB is primarily mediated through interaction with PAR polymers rather than PARP1 (El-Khamisy *et al.*, 2003). This was later supported by Mortusewicz *et al*. and Godon *et al*. whom observed strong reduction of XRCC1 recruitment by inactivation of PARP1s PARylation domain or by use of PARylation inhibitor (Mortusewicz *et al.*, 2007; Godon *et al.*, 2008). Godon *et al*. further demonstrated that PARylation inhibition with 4-AN caused increased PARP1 accumulation in near-UVA mIF and suggested that PARP1 dissociation from SSB was hampered by the lack of PAR polymers causing reduced BER efficiency. Interestingly, our near-UVA mIF assays on HeLa treated with two structurally different PARylation inhibitors differ from these observations (paper 2). While 4-AN treatment induced increased accumulation of PARP1 to near-UVA microirradiated areas, as observed by Godon *et al*., PJ34 did not. PARP1 foci were only observed in PJ34 treated HeLa after irradiation with high dose near-UVA. Our results suggest that the two PARylation inhibitors have diverging effects on PARP1 affinity for DNA, and represented an opportunity to assess effects of PARP1 accumulation on XRCC1 complex recruitment while PARylation is severely hampered (undetectable). Notably, XRCC1, PNKP and POLβ recruitment to high dose near-UVA mIF did not increase as PARP1 accumulation increased in 4-AN treated cells in comparison with

PJ34 treated cells. At low dose near-UVA microirradiation PJ34 treated cells did not produce any detectable PARP1 nor XRCC1 foci, whereas PARP1 and XRCC1 were observed in both untreated and 4-AN treated cells. Our results therefore suggest that while PARP1 is important in recruitment of XRCC1 complexes when DNA damage is below a certain threshold, XRCC1 is probably not recruited through direct interaction with PARP1. As PARylation inhibition only slightly reduced accumulation of XRCC1, PNKP and POLβ our results indicate that PARP1 mainly influences XRCC1 recruitment through indirect mechanisms, such as through its involvement in chromatin relaxation. However, the increased PARP1 accumulation in 4-AN cells was accompanied by PCNA and FEN1 accumulation, indicating that recruitment of repair factors involved in LP BER and other repair pathways are mediated through direct interaction with PARP1. This PARP1 interaction, the lack of detectable PCNA and FEN1 in low dose near-UVA mIF, and the lower LP BER efficiency compared with SP BER of XRCC1 complexes from unstressed HeLa (paper 2) suggest that recruitment of factors involved in, among other pathways, LP BER is triggered when DNA damage levels are above a certain threshold. High levels of damage could increase the probability of BER intermediates and SSB escaping normal processing, exposing them to interactions causing unprocessable 5'-termini such as oxidized 5' dRP (Demple *et al.*, 2002). SSBR in these cases would require factors involved in strand displacement.

XRCC1 has been reported to be ubiquitinated, sumoylated, acetylated, and phosphorylated. XRCC1 ubiquitination is believed to affect XRCC1 expression levels through degradation and is thus probably not relevant to the rapid recruitment responses to DNA damage (Parsons *et al.*, 2008). XRCC1 sumoylation was demonstrated in an *in vitro* SUMO1 substrate screening in 2005 (Gocke *et al.*, 2005). While no specific conclusion towards its effect on BER was made, sumoylation potentially affects intracellular trafficking and protein-protein interactions. Proteins that take part of multiprotein complexes seem to be overrepresented among SUMO targets (reviewed in (Gocke *et al.*, 2005)). Curated mass spectrometry datasets indicate that human XRCC1

contain three acetylated lysine residues (Lys256, Lys260, and Lys271)[5]. These residues are within, or close to, the XRCC1 NLS and within the region reported to interact with glycosylases, APE1, and PCNA. Recently Yamamori *et al.* reported that deacetylation of APE1 influenced its interaction with XRCC1 and the AP-endonuclease activity of XRCC1 multiprotein complexes (Yamamori *et al.*, 2010). However, Yamamori *et al.* did not assess potential contribution of the XRCC1 acetylations. The most prominent PTM of XRCC1 known to date is, as previously mentioned, phosphorylation. In DDR the usual suspects among kinases are ATM, ATR and DNA-PKcs of the PI3K family. Levy *et al.* demonstrated in 2006 that DNA-PKcs phosphorylates XRCC1 at Ser371 within the BRCT1 domain (Levy *et al.*, 2006). Levy *et al.* also showed that IR induced *in vivo* XRCC1 phosphorylation was undetectable after treatment with PI3K inhibitor Wortmannin, and *in vitro* results suggested that DNA-PKcs phosphorylation of Ser371 mediated dissociation of XRCC1 dimers. In 2008 Chou *et al*. reported that Chk2, believed to be mainly activated by ATM but also ATR, phosphorylates XRCC1 at Thr284 *in vitro* possibly mediating XRCC1 interactions with glycosylases (Chou *et al.*, 2008). *In vivo* Thr284 phosphorylation was reported to be induced by base damage and blocked by inhibition of Chk2. As both DNA-PKcs and Chk2 phosphorylation of XRCC1 were reported to be mediated by DNA damaging stress and potentially could influence XRCC1 recruitment through changes in either the PARP1 or DNA glycosylase binding regions we assessed the effects of Wortmannin and Caffeine (PI3K inhibitors) on near-UVA mIF formation as part of the pilot studies for paper 4. While Wortmannin seemed to increase XRCC1 mIF formation Caffeine reduced it (data not shown). The PI3K inhibitor LY294002 induced a slight reduction in XRCC1 mIF (paper 4). Neither Caffeine (data not shown) nor LY294002 (paper 4) produced any detectable reduction in XRCC1 phosphorylations as assessed by Two-dimensional-PAGE in our hands. However, reduction of XRCC1 phosphorylation was observed when treating with Wortmannine (data not shown) in agreement with Levy *et al.* (Levy *et al.*, 2006). The lack of concurrent mIF observations when treating with Wortmannine and Caffeine is probably a result of diverging PI3K specificities and unspecificities of

[5] Uniprot database, search term "p18887" (Human XRCC1)
http://www.uniprot.org/
Last accessed 12th december 2011

the inhibitors. The lack of observed reduction of phosphorylation of XRCC1 when treating with Caffeine or LY194002 is likely a result of lack of sensitivity in our assay. However, the observed reduction of phosphorylation caused by TBB inhibition of CK2 (paper 4) suggest that PI3Ks mediate fewer phosphorylations on XRCC1 than CK2. CK2 phosphorylation between the BRCT domains of XRCC1 has been shown to mediate interaction with the FHA domains of PNKP, APLF and APTX (end-trimming enzymes) and inhibition affects BER activity (Loizou *et al.*, 2004; Luo *et al.*, 2004; Iles *et al.*, 2007). Our observation that CK2 inhibition did not alter XRCC1 mIF formation (paper 4) thus suggests that FHA domain interactions within the BRCT domains do not participate in mediating recruitment of XRCC1 complexes.

x
x
x
x
x
x
x
x
x This paragraph has been removed from the electronic version
x
x
x
x
x
x
x
x
x
x

This page has been removed from the electronic version

References

Akbari, M., M. Otterlei, et al. (2004). "Repair of U/G and U/A in DNA by UNG2-associated repair complexes takes place predominantly by short-patch repair both in proliferating and growth-arrested cells." Nucleic Acids Res **32**(18): 5486-98.

Akbari, M., M. Otterlei, et al. (2007). "Different organization of base excision repair of uracil in DNA in nuclei and mitochondria and selective upregulation of mitochondrial uracil-DNA glycosylase after oxidative stress." Neuroscience **145**(4): 1201-12.

Akbari, M., T. Visnes, et al. (2008). "Mitochondrial base excision repair of uracil and AP sites takes place by single-nucleotide insertion and long-patch DNA synthesis." DNA Repair (Amst) **7**(4): 605-16.

Akbari, M., J. Pena-Diaz, et al. (2009). "Extracts of proliferating and non-proliferating human cells display different base excision pathways and repair fidelity." DNA Repair (Amst) **8**(7): 834-43.

Akbari, M., K. Solvang-Garten, et al. (2010). "Direct interaction between XRCC1 and UNG2 facilitates rapid repair of uracil in DNA by XRCC1 complexes." DNA Repair (Amst) **9**(7): 785-95.

Akbari, M. and H. E. Krokan (2011). "Base excision repair efficiency and mechanism in nuclear extracts are influenced by the ratio between volume of nuclear extraction buffer and nuclei-Implications for comparative studies." Mutat Res.

Allinson, S. L., Dianova, II, et al. (2001). "DNA polymerase beta is the major dRP lyase involved in repair of oxidative base lesions in DNA by mammalian cell extracts." EMBO J **20**(23): 6919-26.

Almeida, K. H. and R. W. Sobol (2007). "A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification." DNA Repair (Amst) **6**(6): 695-711.

Ame, J. C., V. Rolli, et al. (1999). "PARP-2, A novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase." J Biol Chem **274**(25): 17860-8.

Araten, D. J., D. W. Golde, et al. (2005). "A quantitative measurement of the human somatic mutation rate." Cancer Res **65**(18): 8111-7.

Audebert, M., B. Salles, et al. (2004). "Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining." J Biol Chem **279**(53): 55117-26.

Audebert, M., B. Salles, et al. (2006). "Involvement of polynucleotide kinase in a poly(ADP-ribose) polymerase-1-dependent DNA double-strand breaks rejoining pathway." J Mol Biol **356**(2): 257-65.

Bandaru, V., S. Sunkara, et al. (2002). "A novel human DNA glycosylase that removes oxidative DNA damage and is homologous to Escherichia coli endonuclease VIII." DNA Repair (Amst) **1**(7): 517-29.

Barnes, D. E., L. H. Johnston, et al. (1990). "Human DNA ligase I cDNA: cloning and functional expression in Saccharomyces cerevisiae." Proc Natl Acad Sci U S A **87**(17): 6679-83.

Bauer, P. I., K. G. Buki, et al. (1990). "Macromolecular association of ADP-ribosyltransferase and its correlation with enzymic activity." Biochem J **270**(1): 17-26.
Beard, W. A. and S. H. Wilson (2006). "Structure and mechanism of DNA polymerase Beta." Chem Rev **106**(2): 361-82.
Bebenek, K., A. Tissier, et al. (2001). "5'-Deoxyribose phosphate lyase activity of human DNA polymerase iota in vitro." Science **291**(5511): 2156-9.
Beernink, P. T., M. Hwang, et al. (2005). "Specificity of protein interactions mediated by BRCT domains of the XRCC1 DNA repair protein." J Biol Chem **280**(34): 30206-13.
Bekker-Jensen, S., K. Fugger, et al. (2007). "Human Xip1 (C2orf13) is a novel regulator of cellular responses to DNA strand breaks." J Biol Chem **282**(27): 19638-43.
Berkovich, E., R. J. Monnat, Jr., et al. (2007). "Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair." Nat Cell Biol **9**(6): 683-90.
Bernstein, N. K., M. Hammel, et al. (2009). "Mechanism of DNA substrate recognition by the mammalian DNA repair enzyme, Polynucleotide Kinase." Nucleic Acids Res **37**(18): 6161-73.
Bhagwat, A. S., R. J. Sanderson, et al. (1999). "Delayed DNA joining at 3' mismatches by human DNA ligases." Nucleic Acids Res **27**(20): 4028-33.
Boldogh, I., D. Milligan, et al. (2001). "hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence suggesting replication-coupled repair of adenine:8-oxoguanine mispairs." Nucleic Acids Res **29**(13): 2802-9.
Braithwaite, E. K., R. Prasad, et al. (2005). "DNA polymerase lambda mediates a back-up base excision repair activity in extracts of mouse embryonic fibroblasts." J Biol Chem **280**(18): 18469-75.
Brem, R. and J. Hall (2005). "XRCC1 is required for DNA single-strand break repair in human cells." Nucleic Acids Res **33**(8): 2512-20.
Breslin, C. and K. W. Caldecott (2009). "DNA 3'-phosphatase activity is critical for rapid global rates of single-strand break repair following oxidative stress." Mol Cell Biol **29**(17): 4653-62.
Britten, R. J. (2002). "Divergence between samples of chimpanzee and human DNA sequences is 5%, counting indels." Proc Natl Acad Sci U S A **99**(21): 13633-5.
Brunnemann, K. D., L. Genoble, et al. (1987). "Identification and analysis of a new tobacco-specific N-nitrosamine, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol." Carcinogenesis **8**(3): 465-9.
Burkovics, P., V. Szukacsov, et al. (2006). "Human Ape2 protein has a 3'-5' exonuclease activity that acts preferentially on mismatched base pairs." Nucleic Acids Res **34**(9): 2508-15.
Caldecott, K. W., J. D. Tucker, et al. (1995). "Characterization of the XRCC1-DNA ligase III complex in vitro and its absence from mutant hamster cells." Nucleic Acids Res **23**(23): 4836-43.
Caldecott, K. W., S. Aoufouchi, et al. (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 Res **24**(22): 4387-94.

Caldecott, K. W. (2001). "Mammalian DNA single-strand break repair: an X-ra(y)ted affair." Bioessays **23**(5): 447-55.
Caldecott, K. W. (2003). "XRCC1 and DNA strand break repair." DNA Repair (Amst) **2**(9): 955-69.
Caldecott, K. W. (2008). "Single-strand break repair and genetic disease." Nat Rev Genet **9**(8): 619-31.
Caldecott, K. W. (2009). Chromosomal single-strand break repair. The DNA Damage Response: Implications on Cancer Formation and Treatment. K. K. Khanna and Y. Shiloh. Dordrecht, Springer Netherlands**:** 261-284.
Campalans, A., S. Marsin, et al. (2005). "XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair." DNA Repair (Amst) **4**(7): 826-35.
Cann, K. L. and G. Dellaire (2011). "Heterochromatin and the DNA damage response: the need to relax." Biochem Cell Biol **89**(1): 45-60.
Cappelli, E., R. Taylor, et al. (1997). "Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair." J Biol Chem **272**(38): 23970-5.
Chattopadhyay, R., L. Wiederhold, et al. (2006). "Identification and characterization of mitochondrial abasic (AP)-endonuclease in mammalian cells." Nucleic Acids Res **34**(7): 2067-76.
Chen, B., Y. Zhou, et al. (2011). "Polymorphisms of XRCC1 and gastric cancer susceptibility: a meta-analysis." Mol Biol Rep.
Chen, J., A. E. Tomkinson, et al. (1995). "Mammalian DNA ligase III: molecular cloning, chromosomal localization, and expression in spermatocytes undergoing meiotic recombination." Mol Cell Biol **15**(10): 5412-22.
Chou, K. M. and Y. C. Cheng (2002). "An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3' mispaired DNA." Nature **415**(6872): 655-9.
Chou, W. C., H. C. Wang, et al. (2008). "Chk2-dependent phosphorylation of XRCC1 in the DNA damage response promotes base excision repair." EMBO J **27**(23): 3140-50.
Ciccia, A. and S. J. Elledge (2010). "The DNA damage response: making it safe to play with knives." Mol Cell **40**(2): 179-204.
Crick, F. (1974). "The double helix: a personal view." Nature **248**(5451): 766-9.
Cuenda, A. and S. Rousseau (2007). "p38 MAP-kinases pathway regulation, function and role in human diseases." Biochim Biophys Acta **1773**(8): 1358-75.
Cuneo, M. J. and R. E. London (2010). "Oxidation state of the XRCC1 N-terminal domain regulates DNA polymerase beta binding affinity." Proc Natl Acad Sci U S A **107**(15): 6805-10.
Cuneo, M. J., S. A. Gabel, et al. (2011). "The structural basis for partitioning of the XRCC1/DNA ligase III-{alpha} BRCT-mediated dimer complexes." Nucleic Acids Res.
D'Amours, D., S. Desnoyers, et al. (1999). "Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions." Biochem J **342 (Pt 2)**: 249-68.
Dantzer, F., G. de La Rubia, et al. (2000). "Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1." Biochemistry **39**(25): 7559-69.

Date, H., S. Igarashi, et al. (2004). "The FHA domain of aprataxin interacts with the C-terminal region of XRCC1." Biochem Biophys Res Commun **325**(4): 1279-85.
de Murcia, G. and J. Menissier de Murcia (1994). "Poly(ADP-ribose) polymerase: a molecular nick-sensor." Trends Biochem Sci **19**(4): 172-6.
Demple, B. and M. S. DeMott (2002). "Dynamics and diversions in base excision DNA repair of oxidized abasic lesions." Oncogene **21**(58): 8926-34.
Dianov, G., A. Price, et al. (1992). "Generation of single-nucleotide repair patches following excision of uracil residues from DNA." Mol Cell Biol **12**(4): 1605-12.
Dianov, G. L., R. Prasad, et al. (1999). "Role of DNA polymerase beta in the excision step of long patch mammalian base excision repair." J Biol Chem **274**(20): 13741-3.
Dianova, II, V. A. Bohr, et al. (2001). "Interaction of human AP endonuclease 1 with flap endonuclease 1 and proliferating cell nuclear antigen involved in long-patch base excision repair." Biochemistry **40**(42): 12639-44.
Dogliotti, E., P. Fortini, et al. (2001). "The mechanism of switching among multiple BER pathways." Prog Nucleic Acid Res Mol Biol **68**: 3-27.
Douki, T., A. Reynaud-Angelin, et al. (2003). "Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation." Biochemistry **42**(30): 9221-6.
Durocher, D. (2009). DNA damage sensing and signalling. The DNA Damage Response: Implications on Cancer Formation and Treatment. K. K. Khanna and Y. Shiloh. Dordrecht, Springer Netherlands**:** 1-24.
Ehrlich, M., M. A. Gama-Sosa, et al. (1982). "Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells." Nucleic Acids Res **10**(8): 2709-21.
Eissenberg, J. C., R. Ayyagari, et al. (1997). "Mutations in yeast proliferating cell nuclear antigen define distinct sites for interaction with DNA polymerase delta and DNA polymerase epsilon." Mol Cell Biol **17**(11): 6367-78.
El-Khamisy, S. F., M. Masutani, et al. (2003). "A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage." Nucleic Acids Res **31**(19): 5526-33.
El-Khamisy, S. F., G. M. Saifi, et al. (2005). "Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1." Nature **434**(7029): 108-13.
Ellenberger, T. and A. E. Tomkinson (2008). "Eukaryotic DNA ligases: structural and functional insights." Annu Rev Biochem **77**: 313-38.
Evensen, G. and E. Seeberg (1982). "Adaptation to alkylation resistance involves the induction of a DNA glycosylase." Nature **296**(5859): 773-5.
Falck, J., J. Coates, et al. (2005). "Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage." Nature **434**(7033): 605-11.
Fan, J., M. Otterlei, et al. (2004). "XRCC1 co-localizes and physically interacts with PCNA." Nucleic Acids Res **32**(7): 2193-201.
Ferguson, L. R. (2010). "Chronic inflammation and mutagenesis." Mutat Res **690**(1-2): 3-11.
Fortini, P., E. Parlanti, et al. (1999). "The type of DNA glycosylase determines the base excision repair pathway in mammalian cells." J Biol Chem **274**(21): 15230-6.
Friedberg, E. C., W. Siede, et al. (2006). DNA repair and mutagenesis. Washington, D.C., ASM Press.

Friedman, J. I. and J. T. Stivers (2010). "Detection of damaged DNA bases by DNA glycosylase enzymes." Biochemistry **49**(24): 4957-67.
Frosina, G., P. Fortini, et al. (1996). "Two pathways for base excision repair in mammalian cells." J Biol Chem **271**(16): 9573-8.
Frouin, I., G. Maga, et al. (2003). "Human proliferating cell nuclear antigen, poly(ADP-ribose) polymerase-1, and p21waf1/cip1. A dynamic exchange of partners." J Biol Chem **278**(41): 39265-8.
Gagne, J. P., M. Isabelle, et al. (2008). "Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes." Nucleic Acids Res **36**(22): 6959-76.
Gaiddon, C., N. C. Moorthy, et al. (1999). "Ref-1 regulates the transactivation and pro-apoptotic functions of p53 in vivo." EMBO J **18**(20): 5609-21.
Gao, Y., S. Katyal, et al. (2011). "DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair." Nature **471**(7337): 240-4.
Gary, R., K. Kim, et al. (1999). "Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair." J Biol Chem **274**(7): 4354-63.
Girard, P. M., S. Francesconi, et al. (2011). "UVA-induced damage to DNA and proteins: direct versus indirect photochemical processes." Cost Chemistry Cm0603-Melusyn Joint Meeting: Damages Induced in Biomolecules by Low and High Energy Radiations **261**.
Gocke, C. B., H. Yu, et al. (2005). "Systematic identification and analysis of mammalian small ubiquitin-like modifier substrates." J Biol Chem **280**(6): 5004-12.
Godon, C., F. P. Cordelieres, et al. (2008). "PARP inhibition versus PARP-1 silencing: different outcomes in terms of single-strand break repair and radiation susceptibility." Nucleic Acids Res **36**(13): 4454-64.
Gsur, A., K. Bernhart, et al. (2011). "No association of XRCC1 polymorphisms Arg194Trp and Arg399Gln with colorectal cancer risk." Cancer Epidemiol.
Gu, H., J. D. Marth, et al. (1994). "Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting." Science **265**(5168): 103-6.
Hagen, L., B. Kavli, et al. (2008). "Cell cycle-specific UNG2 phosphorylations regulate protein turnover, activity and association with RPA." EMBO J **27**(1): 51-61.
Haince, J. F., S. Kozlov, et al. (2007). "Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents." J Biol Chem **282**(22): 16441-53.
Hanif, I. M., M. A. Shazib, et al. (2010). "Casein Kinase II: an attractive target for anti-cancer drug design." Int J Biochem Cell Biol **42**(10): 1602-5.
Hanssen-Bauer, A., K. Solvang-Garten, et al. (2011). "XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage." Environ Mol Mutagen **52**(8): 623-35.
Harrington, J. J. and M. R. Lieber (1994). "The characterization of a mammalian DNA structure-specific endonuclease." EMBO J **13**(5): 1235-46.
Hazra, T. K., T. Izumi, et al. (2002a). "Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA." Proc Natl Acad Sci U S A **99**(6): 3523-8.

Hazra, T. K., Y. W. Kow, et al. (2002b). "Identification and characterization of a novel human DNA glycosylase for repair of cytosine-derived lesions." J Biol Chem **277**(34): 30417-20.
Heale, J. T., A. R. Ball, Jr., et al. (2006). "Condensin I interacts with the PARP-1-XRCC1 complex and functions in DNA single-strand break repair." Mol Cell **21**(6): 837-48.
Hochegger, H., D. Dejsuphong, et al. (2006). "Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells." EMBO J **25**(6): 1305-14.
Hoffmann, D. and S. S. Hecht (1985). "Nicotine-derived N-nitrosamines and tobacco-related cancer: current status and future directions." Cancer Res **45**(3): 935-44.
Hofmann, K. and P. Bucher (1995). "The FHA domain: a putative nuclear signalling domain found in protein kinases and transcription factors." Trends Biochem Sci **20**(9): 347-9.
Huang, J., J. Zhang, et al. (2011). "The Arg194Trp polymorphism in the XRCC1 gene and cancer risk in Chinese Mainland population: a meta-analysis." Mol Biol Rep.
Iles, N., S. Rulten, et al. (2007). "APLF (C2orf13) is a novel human protein involved in the cellular response to chromosomal DNA strand breaks." Mol Cell Biol **27**(10): 3793-803.
Imai, K., G. Slupphaug, et al. (2003). "Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination." Nat Immunol **4**(10): 1023-8.
Isabelle, M., X. Moreel, et al. (2010). "Investigation of PARP-1, PARP-2, and PARG interactomes by affinity-purification mass spectrometry." Proteome Sci **8**: 22.
Jaenisch, R. and A. Bird (2003). "Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals." Nat Genet **33 Suppl**: 245-54.
Jazayeri, A., J. Falck, et al. (2006). "ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks." Nat Cell Biol **8**(1): 37-45.
Jiang, J., X. Liang, et al. (2010). "DNA repair gene X-ray repair cross complementing group 1 Arg194Trp polymorphism on the risk of lung cancer: a meta-analysis on 22 studies." J Thorac Oncol **5**(11): 1741-7.
Kaguni, L. S. (2004). "DNA polymerase gamma, the mitochondrial replicase." Annu Rev Biochem **73**: 293-320.
Kanno, S., H. Kuzuoka, et al. (2007). "A novel human AP endonuclease with conserved zinc-finger-like motifs involved in DNA strand break responses." EMBO J **26**(8): 2094-103.
Karimi-Busheri, F. and M. Weinfeld (1997). "Purification and substrate specificity of polydeoxyribonucleotide kinases isolated from calf thymus and rat liver." J Cell Biochem **64**(2): 258-72.
Karran, P., T. Lindahl, et al. (1980). "Escherichia coli mutants deficient in 3-methyladenine-DNA glycosylase." J Mol Biol **140**(1): 101-27.
Kavli, B., O. Sundheim, et al. (2002). "hUNG2 is the major repair enzyme for removal of uracil from U:A matches, U:G mismatches, and U in single-stranded DNA, with hSMUG1 as a broad specificity backup." J Biol Chem **277**(42): 39926-36.

Kavli, B., S. Andersen, et al. (2005). "B cells from hyper-IgM patients carrying UNG mutations lack ability to remove uracil from ssDNA and have elevated genomic uracil." J Exp Med **201**(12): 2011-21.
Kedar, P. S., S. J. Kim, et al. (2002). "Direct interaction between mammalian DNA polymerase beta and proliferating cell nuclear antigen." J Biol Chem **277**(34): 31115-23.
Khodyreva, S. N., R. Prasad, et al. (2010). "Apurinic/apyrimidinic (AP) site recognition by the 5'-dRP/AP lyase in poly(ADP-ribose) polymerase-1 (PARP-1)." Proc Natl Acad Sci U S A **107**(51): 22090-5.
Kong, X., S. K. Mohanty, et al. (2009). "Comparative analysis of different laser systems to study cellular responses to DNA damage in mammalian cells." Nucleic Acids Res **37**(9): e68.
Kubota, Y., R. A. Nash, et al. (1996). "Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein." EMBO J **15**(23): 6662-70.
Kubota, Y. and S. Horiuchi (2003). "Independent roles of XRCC1's two BRCT motifs in recovery from methylation damage." DNA Repair (Amst) **2**(4): 407-15.
Lakshmipathy, U. and C. Campbell (1999). "The human DNA ligase III gene encodes nuclear and mitochondrial proteins." Mol Cell Biol **19**(5): 3869-76.
Lakshmipathy, U. and C. Campbell (2000). "Mitochondrial DNA ligase III function is independent of Xrcc1." Nucleic Acids Res **28**(20): 3880-6.
Lan, L., S. Nakajima, et al. (2004). "In situ analysis of repair processes for oxidative DNA damage in mammalian cells." Proc Natl Acad Sci U S A **101**(38): 13738-43.
Larsen, E., C. Gran, et al. (2003). "Proliferation failure and gamma radiation sensitivity of Fen1 null mutant mice at the blastocyst stage." Mol Cell Biol **23**(15): 5346-53.
Lavrik, O. I., R. Prasad, et al. (2001). "Photoaffinity labeling of mouse fibroblast enzymes by a base excision repair intermediate. Evidence for the role of poly(ADP-ribose) polymerase-1 in DNA repair." J Biol Chem **276**(27): 25541-8.
Lebedeva, N. A., N. I. Rechkunova, et al. (2011). "AP-site cleavage activity of tyrosyl-DNA phosphodiesterase 1." FEBS Lett **585**(4): 683-6.
Leonhardt, H., H. P. Rahn, et al. (2000). "Dynamics of DNA replication factories in living cells." J Cell Biol **149**(2): 271-80.
Leppard, J. B., Z. Dong, et al. (2003). "Physical and functional interaction between DNA ligase IIIalpha and poly(ADP-Ribose) polymerase 1 in DNA single-strand break repair." Mol Cell Biol **23**(16): 5919-27.
Levin, D. S., W. Bai, et al. (1997). "An interaction between DNA ligase I and proliferating cell nuclear antigen: implications for Okazaki fragment synthesis and joining." Proc Natl Acad Sci U S A **94**(24): 12863-8.
Levy, N., A. Martz, et al. (2006). "XRCC1 is phosphorylated by DNA-dependent protein kinase in response to DNA damage." Nucleic Acids Res **34**(1): 32-41.
Li, H., T. C. Ha, et al. (2009). "XRCC1 gene polymorphisms and breast cancer risk in different populations: a meta-analysis." Breast **18**(3): 183-91.
Lindahl, T. (1993). "Instability and decay of the primary structure of DNA." Nature **362**(6422): 709-15.

Liu, Y., W. A. Beard, et al. (2005). "DNA polymerase beta and flap endonuclease 1 enzymatic specificities sustain DNA synthesis for long patch base excision repair." J Biol Chem **280**(5): 3665-74.
Loizou, J. I., S. F. El-Khamisy, et al. (2004). "The protein kinase CK2 facilitates repair of chromosomal DNA single-strand breaks." Cell **117**(1): 17-28.
Longley, M. J., R. Prasad, et al. (1998). "Identification of 5'-deoxyribose phosphate lyase activity in human DNA polymerase gamma and its role in mitochondrial base excision repair in vitro." Proc Natl Acad Sci U S A **95**(21): 12244-8.
Loor, G., S. J. Zhang, et al. (1997). "Identification of DNA replication and cell cycle proteins that interact with PCNA." Nucleic Acids Res **25**(24): 5041-6.
Luo, H., D. W. Chan, et al. (2004). "A new XRCC1-containing complex and its role in cellular survival of methyl methanesulfonate treatment." Mol Cell Biol **24**(19): 8356-65.
Maga, G. and U. Hubscher (2003). "Proliferating cell nuclear antigen (PCNA): a dancer with many partners." J Cell Sci **116**(Pt 15): 3051-60.
Mahaney, B. L., K. Meek, et al. (2009). "Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining." Biochem J **417**(3): 639-50.
Mani, R. S., F. Karimi-Busheri, et al. (2004). "Biophysical characterization of human XRCC1 and its binding to damaged and undamaged DNA." Biochemistry **43**(51): 16505-14.
Marenstein, D. R., M. K. Chan, et al. (2003). "Substrate specificity of human endonuclease III (hNTH1). Effect of human APE1 on hNTH1 activity." J Biol Chem **278**(11): 9005-12.
Marintchev, A., M. A. Mullen, et al. (1999). "Solution structure of the single-strand break repair protein XRCC1 N-terminal domain." Nat Struct Biol **6**(9): 884-93.
Marnett, L. J. and P. C. Burcham (1993). "Endogenous DNA adducts: potential and paradox." Chem Res Toxicol **6**(6): 771-85.
Marsin, S., A. E. Vidal, et al. (2003). "Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1." J Biol Chem **278**(45): 44068-74.
Masson, M., C. Niedergang, et al. (1998). "XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage." Mol Cell Biol **18**(6): 3563-71.
Matsuoka, S., B. A. Ballif, et al. (2007). "ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage." Science **316**(5828): 1160-6.
McCulloch, S. D. and T. A. Kunkel (2008). "The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases." Cell Res **18**(1): 148-61.
McNeill, D. R., P. C. Lin, et al. (2011). "XRCC1 haploinsufficiency in mice has little effect on aging, but adversely modifies exposure-dependent susceptibility." Nucleic Acids Res.
Menissier de Murcia, J., M. Ricoul, et al. (2003). "Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse." EMBO J **22**(9): 2255-63.

Mol, C. D., T. Izumi, et al. (2000). "DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination [corrected]." Nature **403**(6768): 451-6.
Moldovan, G. L., B. Pfander, et al. (2007). "PCNA, the maestro of the replication fork." Cell **129**(4): 665-79.
Moller, H., J. Landt, et al. (1989). "Endogenous nitrosation in relation to nitrate exposure from drinking water and diet in a Danish rural population." Cancer Res **49**(11): 3117-21.
Mortusewicz, O., U. Rothbauer, et al. (2006). "Differential recruitment of DNA Ligase I and III to DNA repair sites." Nucleic Acids Res **34**(12): 3523-32.
Mortusewicz, O., J. C. Ame, et al. (2007). "Feedback-regulated poly(ADP-ribosyl)ation by PARP-1 is required for rapid response to DNA damage in living cells." Nucleic Acids Res **35**(22): 7665-75.
Moser, J., H. Kool, et al. (2007). "Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner." Mol Cell **27**(2): 311-23.
Mouret, S., C. Baudouin, et al. (2006). "Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation." Proc Natl Acad Sci U S A **103**(37): 13765-70.
Murante, R. S., L. Huang, et al. (1994). "The calf 5'- to 3'-exonuclease is also an endonuclease with both activities dependent on primers annealed upstream of the point of cleavage." J Biol Chem **269**(2): 1191-6.
Nagy, Z. and E. Soutoglou (2009). "DNA repair: easy to visualize, difficult to elucidate." Trends Cell Biol **19**(11): 617-29.
Nash, R. A., K. W. Caldecott, et al. (1997). "XRCC1 protein interacts with one of two distinct forms of DNA ligase III." Biochemistry **36**(17): 5207-11.
Nilsen, H., M. Otterlei, et al. (1997). "Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the UNG gene." Nucleic Acids Res **25**(4): 750-5.
Nilsen, H., K. S. Steinsbekk, et al. (2000). "Analysis of uracil-DNA glycosylases from the murine Ung gene reveals differential expression in tissues and in embryonic development and a subcellular sorting pattern that differs from the human homologues." Nucleic Acids Res **28**(12): 2277-85.
Nishi, T., N. Shimizu, et al. (2002). "Spatial redox regulation of a critical cysteine residue of NF-kappa B in vivo." J Biol Chem **277**(46): 44548-56.
Nouspikel, T. (2009). "DNA repair in mammalian cells : Nucleotide excision repair: variations on versatility." Cell Mol Life Sci **66**(6): 994-1009.
Ochs, K., R. W. Sobol, et al. (1999). "Cells deficient in DNA polymerase beta are hypersensitive to alkylating agent-induced apoptosis and chromosomal breakage." Cancer Res **59**(7): 1544-51.
Ohshima, H., M. Tatemichi, et al. (2003). "Chemical basis of inflammation-induced carcinogenesis." Arch Biochem Biophys **417**(1): 3-11.
Okano, S., L. Lan, et al. (2003). "Spatial and temporal cellular responses to single-strand breaks in human cells." Mol Cell Biol **23**(11): 3974-81.
Otterlei, M., E. Warbrick, et al. (1999). "Post-replicative base excision repair in replication foci." EMBO J **18**(13): 3834-44.

Oyama, M., M. Wakasugi, et al. (2004). "Human NTH1 physically interacts with p53 and proliferating cell nuclear antigen." Biochem Biophys Res Commun **321**(1): 183-91.
Park, C. B. and N. G. Larsson (2011). "Mitochondrial DNA mutations in disease and aging." J Cell Biol **193**(5): 809-18.
Parker, A., Y. Gu, et al. (2001). "Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair." J Biol Chem **276**(8): 5547-55.
Parlanti, E., G. Locatelli, et al. (2007). "Human base excision repair complex is physically associated to DNA replication and cell cycle regulatory proteins." Nucleic Acids Res **35**(5): 1569-77.
Parsons, J. L., Dianova, II, et al. (2004). "APE1 is the major 3'-phosphoglycolate activity in human cell extracts." Nucleic Acids Res **32**(12): 3531-6.
Parsons, J. L., P. S. Tait, et al. (2008). "CHIP-mediated degradation and DNA damage-dependent stabilization regulate base excision repair proteins." Mol Cell **29**(4): 477-87.
Pascal, J. M., P. J. O'Brien, et al. (2004). "Human DNA ligase I completely encircles and partially unwinds nicked DNA." Nature **432**(7016): 473-8.
Pascucci, B., G. Maga, et al. (2002). "Reconstitution of the base excision repair pathway for 7,8-dihydro-8-oxoguanine with purified human proteins." Nucleic Acids Res **30**(10): 2124-30.
Pesole, G., C. Gissi, et al. (1999). "Nucleotide substitution rate of mammalian mitochondrial genomes." J Mol Evol **48**(4): 427-34.
Pleschke, J. M., H. E. Kleczkowska, et al. (2000). "Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins." J Biol Chem **275**(52): 40974-80.
Podlutsky, A. J., Dianova, II, et al. (2001a). "Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA." EMBO J **20**(6): 1477-82.
Podlutsky, A. J., Dianova, II, et al. (2001b). "DNA synthesis and dRPase activities of polymerase beta are both essential for single-nucleotide patch base excision repair in mammalian cell extracts." Biochemistry **40**(3): 809-13.
Povey, A. C., A. F. Badawi, et al. (2002). "DNA alkylation and repair in the large bowel: animal and human studies." J Nutr **132**(11 Suppl): 3518S-3521S.
Prasad, R., W. A. Beard, et al. (1998). "Functional analysis of the amino-terminal 8-kDa domain of DNA polymerase beta as revealed by site-directed mutagenesis. DNA binding and 5'-deoxyribose phosphate lyase activities." J Biol Chem **273**(18): 11121-6.
Prasad, R., O. I. Lavrik, et al. (2001). "DNA polymerase beta -mediated long patch base excision repair. Poly(ADP-ribose)polymerase-1 stimulates strand displacement DNA synthesis." J Biol Chem **276**(35): 32411-4.
Prasad, R., M. J. Longley, et al. (2009). "Human DNA polymerase theta possesses 5'-dRP lyase activity and functions in single-nucleotide base excision repair in vitro." Nucleic Acids Res **37**(6): 1868-77.
Prasad, R., D. D. Shock, et al. (2010). "Substrate channeling in mammalian base excision repair pathways: passing the baton." J Biol Chem **285**(52): 40479-88.
Puebla-Osorio, N., D. B. Lacey, et al. (2006). "Early embryonic lethality due to targeted inactivation of DNA ligase III." Mol Cell Biol **26**(10): 3935-41.

Rada, C., G. T. Williams, et al. (2002). "Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice." Curr Biol **12**(20): 1748-55.
Ravanat, J. L., T. Douki, et al. (2001). "Direct and indirect effects of UV radiation on DNA and its components." J Photochem Photobiol B **63**(1-3): 88-102.
Reinhardt, H. C., A. S. Aslanian, et al. (2007). "p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage." Cancer Cell **11**(2): 175-89.
Robertson, A. B., A. Klungland, et al. (2009). "DNA repair in mammalian cells: Base excision repair: the long and short of it." Cell Mol Life Sci **66**(6): 981-93.
Rouleau, M., A. Patel, et al. (2010). "PARP inhibition: PARP1 and beyond." Nat Rev Cancer **10**(4): 293-301.
Rulten, S. L., A. E. Fisher, et al. (2011). "PARP-3 and APLF function together to accelerate nonhomologous end-joining." Mol Cell **41**(1): 33-45.
Rydberg, B. and T. Lindahl (1982). "Nonenzymatic methylation of DNA by the intracellular methyl group donor S-adenosyl-L-methionine is a potentially mutagenic reaction." EMBO J **1**(2): 211-6.
Sakasai, R. and R. S. Tibbetts (2009). Cell cycle regulation and DNA damage. The DNA Damage Response: Implications on Cancer Formation and Treatment. K. K. Khanna and Y. Shiloh. Dordrecht, Springer Netherlands**:** 81-107.
Scanlan, R. A. (1983). "Formation and occurrence of nitrosamines in food." Cancer Res **43**(5 Suppl): 2435s-2440s.
Schreiber, V., J. C. Ame, et al. (2002). "Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1." J Biol Chem **277**(25): 23028-36.
Schreiber, V., F. Dantzer, et al. (2006). "Poly(ADP-ribose): novel functions for an old molecule." Nat Rev Mol Cell Biol **7**(7): 517-28.
Shiotani, B. and L. Zou (2009). "Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks." Mol Cell **33**(5): 547-58.
Shrivastav, N., D. Li, et al. (2010). "Chemical biology of mutagenesis and DNA repair: cellular responses to DNA alkylation." Carcinogenesis **31**(1): 59-70.
Simsek, D., A. Furda, et al. (2011). "Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair." Nature **471**(7337): 245-8.
Sleeth, K. M., R. L. Robson, et al. (2004). "Exchangeability of mammalian DNA ligases between base excision repair pathways." Biochemistry **43**(40): 12924-30.
Slupphaug, G., C. D. Mol, et al. (1996). "A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA." Nature **384**(6604): 87-92.
Sobol, R. W., R. Prasad, et al. (2000). "The lyase activity of the DNA repair protein beta-polymerase protects from DNA-damage-induced cytotoxicity." Nature **405**(6788): 807-10.
Stokes, M. P., J. Rush, et al. (2007). "Profiling of UV-induced ATM/ATR signaling pathways." Proc Natl Acad Sci U S A **104**(50): 19855-60.
Stucki, M., B. Pascucci, et al. (1998). "Mammalian base excision repair by DNA polymerases delta and epsilon." Oncogene **17**(7): 835-43.

Suh, D., D. M. Wilson, 3rd, et al. (1997). "3'-phosphodiesterase activity of human apurinic/apyrimidinic endonuclease at DNA double-strand break ends." Nucleic Acids Res **25**(12): 2495-500.
Sukhanova, M., S. Khodyreva, et al. (2010). "Poly(ADP-ribose) polymerase 1 regulates activity of DNA polymerase beta in long patch base excision repair." Mutat Res **685**(1-2): 80-9.
Sung, J. S. and B. Demple (2006). "Roles of base excision repair subpathways in correcting oxidized abasic sites in DNA." FEBS J **273**(8): 1620-9.
Szczesny, B., A. W. Tann, et al. (2008). "Long patch base excision repair in mammalian mitochondrial genomes." J Biol Chem **283**(39): 26349-56.
Taylor, R. M., B. Wickstead, et al. (1998). "Role of a BRCT domain in the interaction of DNA ligase III-alpha with the DNA repair protein XRCC1." Curr Biol **8**(15): 877-80.
Taylor, R. M., A. Thistlethwaite, et al. (2002). "Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair." Mol Cell Biol **22**(8): 2556-63.
Tebbs, R. S., M. L. Flannery, et al. (1999). "Requirement for the Xrcc1 DNA base excision repair gene during early mouse development." Dev Biol **208**(2): 513-29.
Tebbs, R. S., L. H. Thompson, et al. (2003). "Rescue of Xrcc1 knockout mouse embryo lethality by transgene-complementation." DNA Repair (Amst) **2**(12): 1405-17.
Tell, G., D. Fantini, et al. (2010). "Understanding different functions of mammalian AP endonuclease (APE1) as a promising tool for cancer treatment." Cell Mol Life Sci **67**(21): 3589-608.
Thompson, L. H., K. W. Brookman, et al. (1990). "Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange." Mol Cell Biol **10**(12): 6160-71.
Thompson, L. H. and M. G. West (2000). "XRCC1 keeps DNA from getting stranded." Mutat Res **459**(1): 1-18.
Vidal, A. E., S. Boiteux, et al. (2001). "XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions." EMBO J **20**(22): 6530-9.
Vineis, P., M. Manuguerra, et al. (2009). "A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility." J Natl Cancer Inst **101**(1): 24-36.
Wagner, E. F. and A. R. Nebreda (2009). "Signal integration by JNK and p38 MAPK pathways in cancer development." Nat Rev Cancer **9**(8): 537-49.
Wang, M., W. Wu, et al. (2006). "PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways." Nucleic Acids Res **34**(21): 6170-82.
Wang, W., P. Brandt, et al. (2004). "The human Rad9-Rad1-Hus1 checkpoint complex stimulates flap endonuclease 1." Proc Natl Acad Sci U S A **101**(48): 16762-7.
Warmerdam, D. O. and R. Kanaar (2010). "Dealing with DNA damage: relationships between checkpoint and repair pathways." Mutat Res **704**(1-3): 2-11.
Wei, Y. F., P. Robins, et al. (1995). "Molecular cloning and expression of human cDNAs encoding a novel DNA ligase IV and DNA ligase III, an enzyme active in DNA repair and recombination." Mol Cell Biol **15**(6): 3206-16.

Weibrecht, I., K. J. Leuchowius, et al. (2010). "Proximity ligation assays: a recent addition to the proteomics toolbox." Expert Rev Proteomics **7**(3): 401-9.
Whitehouse, C. J., R. M. Taylor, et al. (2001). "XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair." Cell **104**(1): 107-17.
Wiederhold, L., J. B. Leppard, et al. (2004). "AP endonuclease-independent DNA base excision repair in human cells." Mol Cell **15**(2): 209-20.
Wilson, D. M., 3rd, M. Takeshita, et al. (1995). "Incision activity of human apurinic endonuclease (Ape) at abasic site analogs in DNA." J Biol Chem **270**(27): 16002-7.
Witko-Sarsat, V., J. Mocek, et al. (2010). "Proliferating cell nuclear antigen acts as a cytoplasmic platform controlling human neutrophil survival." J Exp Med **207**(12): 2631-45.
Wood, R. D., M. Mitchell, et al. (2005). "Human DNA repair genes, 2005." Mutat Res **577**(1-2): 275-83.
Xanthoudakis, S. and T. Curran (1992). "Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity." EMBO J **11**(2): 653-65.
Xanthoudakis, S., R. J. Smeyne, et al. (1996). "The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice." Proc Natl Acad Sci U S A **93**(17): 8919-23.
Xia, L., L. Zheng, et al. (2005). "Human 3-methyladenine-DNA glycosylase: effect of sequence context on excision, association with PCNA, and stimulation by AP endonuclease." J Mol Biol **346**(5): 1259-74.
Xue, H., P. Ni, et al. (2011). "X-ray repair cross-complementing group 1 (XRCC1) genetic polymorphisms and gastric cancer risk: A HuGE review and meta-analysis." Am J Epidemiol **173**(4): 363-75.
Yamamori, T., J. DeRicco, et al. (2010). "SIRT1 deacetylates APE1 and regulates cellular base excision repair." Nucleic Acids Res **38**(3): 832-45.
Yamtich, J. and J. B. Sweasy (2010). "DNA polymerase family X: function, structure, and cellular roles." Biochim Biophys Acta **1804**(5): 1136-50.
Yelamos, J., V. Schreiber, et al. (2008). "Toward specific functions of poly(ADP-ribose) polymerase-2." Trends Mol Med **14**(4): 169-78.
Zhang, P., J. Y. Mo, et al. (1999). "Direct interaction of proliferating cell nuclear antigen with the p125 catalytic subunit of mammalian DNA polymerase delta." J Biol Chem **274**(38): 26647-53.
Zheng, L., H. Dai, et al. (2007). "Disruption of the FEN-1/PCNA interaction results in DNA replication defects, pulmonary hypoplasia, pancytopenia, and newborn lethality in mice." Mol Cell Biol **27**(8): 3176-86.
Zheng, L., J. Jia, et al. (2011). "Functional regulation of FEN1 nuclease and its link to cancer." Nucleic Acids Res **39**(3): 781-94.
Zou, L. and S. J. Elledge (2003). "Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes." Science **300**(5625): 1542-8.

PAPER 1

DNA Repair 9 (2010) 785–795

Contents lists available at ScienceDirect

DNA Repair

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

Direct interaction between XRCC1 and UNG2 facilitates rapid repair of uracil in DNA by XRCC1 complexes

Mansour Akbari[a], Karin Solvang-Garten[a,1], Audun Hanssen-Bauer[a,1], Nora Valeska Lieske[a], Henrik Sahlin Pettersen[a], Grete Klippenvåg Pettersen[a], David M. Wilson III[b], Hans E. Krokan[a], Marit Otterlei[a,*]

[a] Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, N-7489 Trondheim, Norway
[b] Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, USA

ARTICLE INFO

Article history:
Received 27 October 2009
Received in revised form 31 March 2010
Accepted 1 April 2010
Available online 13 May 2010

Keywords:
Base excision repair
XRCC1
UNG2
DNA repair complexes
Replication associated repair

ABSTRACT

Uracil-DNA glycosylase, UNG2, interacts with PCNA and initiates post-replicative base excision repair (BER) of uracil in DNA. The DNA repair protein XRCC1 also co-localizes and physically interacts with PCNA. However, little is known about whether UNG2 and XRCC1 directly interact and participate in a same complex for repair of uracil in replication foci. Here, we examine localization pattern of these proteins in live and fixed cells and show that UNG2 and XRCC1 are likely in a common complex in replication foci. Using pull-down experiments we demonstrate that UNG2 directly interacts with the nuclear localization signal-region (NLS) of XRCC1. Western blot and functional analysis of immunoprecipitates from whole cell extracts prepared from S-phase enriched cells demonstrate the presence of XRCC1 complexes that contain UNG2 in addition to separate XRCC1 and UNG2 associated complexes with distinct repair features. XRCC1 complexes performed complete repair of uracil with higher efficacy than UNG2 complexes. Based on these results, we propose a model for a functional role of XRCC1 in replication associated BER of uracil.

1. Introduction

Uracil may be introduced in DNA by two distinct mechanisms. One is by hydrolytic deamination of cytosine that generates some 70–200 uracil bases per day in DNA [1]. This deamination results in pre-mutagenic U:G mispairs, which unless repaired, give rise to C:G to T:A transitions upon replication. The other mechanism, which probably is quantitatively a more important source of uracil in DNA, is the incorporation of dUMP in place of dTMP during replication. This results in U:A base pairs that can perturb transcription [2,3] or possibly be mutagenic due to erroneous BER and/or enhanced levels of AP site [4,5].

Base excision repair (BER) of genomic uracil is initiated by one of four uracil-DNA glycosylases (UDG) found in mammalian cells: UNG2, SMUG1, TDG and MBD4 [6]. UNG2 removes uracil from U:A base pairs, and is the only UDG known to interact with PCNA and to localize in replication foci [7,8]. UNG deficiency perturbs normal immune response in humans [9] and mice [10] and contributes to the accumulation of mutations in mice [11].

BER is a multi-step process and can be reconstituted with a limited number of proteins. However, in recent years, a number of proteins have been identified that are not strictly required for BER in vitro, yet exert important effects on cellular BER effectiveness, mainly through protein–protein interactions and post-translational modification [12]. PCNA and XRCC1 are two key proteins that seemingly provide platforms for repair protein interactions. XRCC1 was originally identified as a factor involved in single-strand break DNA repair and cells deficient in XRCC1 show elevated levels of sister chromatid exchange [13]. XRCC1 interacts with a number of proteins known to participate in BER including: APE1 [14], POLβ [15], Lig III [16], PCNA [17], poly (ADP-ribose) polymerase 1 (PARP-1) [18], PARP-2 [19], polynucleotide kinase (PNK) [20] and the DNA glycosylases OGG1 [21], MPG, NEIL1 and hNTH1 [22].

The DNA glycosylases MPG, UNG2 and human MutY homolog (MYH) interact with PCNA through their PCNA binding motif (PIP-box) and co-localize with PCNA in replication foci [8,23–25]. UNG2 is responsible for post-replicative removal of mis-incorporated dUMP during replication [8]. Similar to UNG2, MYH interacts with PCNA and initiates post-replicative repair of adenine from A:8-oxoG mispairs in newly synthesized DNA [23,26]. While these observations suggest the presence of post-replicative BER, little is known about the overall organization of BER during replication.

* Corresponding author. Tel.: +47 72573075; fax: +47 72576400.
E-mail address: marit.otterlei@ntnu.no (M. Otterlei).
[1] These authors contributed equally to the work.

1568-7864/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2010.04.002

XRCC1 co-localizes and directly interacts with PCNA [17] and UNG2 immunoprecipitates containing XRCC1 were found to be capable of complete short patch and long patch BER [27]. However, the extent of protein–protein interactions and whether XRCC1 and UNG2 directly interact or are part of a large complex(es) was not elucidated.

Our aim for conducting this study was to investigate whether repair of uracil in DNA in S-phase cells takes place via XRCC1 mediated protein complexes. We show that XRCC1 and UNG2 co-localize in replication foci and are in close proximity of each other. Using a pull-down assay we show that UNG2 directly interacts with the NLS region of XRCC1. Extracts from synchronised cells expressing EYFP fusions of UNG2 and XRCC1 and use of tag specific antibodies, allowed us to directly compare the biochemical activities and protein content of XRCC1 and UNG2 immunoprecipitates from S-phase cells. Our results provide evidence for the presence of XRCC1 complexes containing UNG2 in addition to separate XRCC1 and UNG2 associated complexes with distinct repair characteristics. We propose a model for a role of XRCC1 in repair of uracil in replication foci.

2. Materials and methods

2.1. Chemicals and antibodies

Synthetic oligonucleotides were from Eurogentech (Belgium), [α-^{33}P]dTTP and [α-^{33}P]dCTP (3000 Ci/mmol) were from Amersham Biosciences, restriction enzymes from New England BioLabs, paramagnetic protein-A beads from Dynal (Norway). Phenylmethyl sulfonyl fluoride (PMSF) and Complete protease inhibitor were from Roche. Antibody against (α)-POLβ (ab3181), α-DNA Lig I (ab615), α-DNA Lig III (ab587), α-APE-1 (ab194), α-XRCC1 (ab1838 (mouse) and abcam 11429 (rabbit)), α-PARP-1 (ab18376), α-FLAG (Sigma) and α-GFP (ab290, Abcam, UK), α-GFP (in house, only used for IP). Both α-GFPs also recognize the other GFP variants (ECFP and EYFP). Other antibodies used were α-PNK (MAB-005, Cytostore, USA), α-PCNA (PC10, Santa Cruz, USA), α-Fen-1 (Betyl, USA), and in-house affinity purified rabbit polyclonal antibodies raised against UNG2 [28]. Secondary antibodies: polyclonal rabbit α-mouse IgG/HRP or peroxidase-labeled polyclonal swine α-rabbit IgG) were from Dako Cytomation (Denmark), Alexa fluor 532 goat-α-mouse IgG and Alexa Fluor 647 goat-α-rabbit IgG (Invitrogen). IgGs were crosslinked to protein-A magnetic beads according to procedure (New England Biolabs).

2.2. Cloning of fusion constructs

pEC/YFP-PCNA, HcRed-PCNA, pXRCC1-EYFP have been described [17,29,30]. The UNG2-EYFP construct is a modified version of the ProA-UNG2-EYFP construct from which UNG2-EYFP transcription is regulated by its own promote [7]. A 3× FLAG sequence is cloned in front of UNG2-EYFP in the EcoRI blunted/KpnI site, thus the FLAG sequence is separated from the N-terminus of UNG2 by a 84 bp linker.

Hamster Xrcc1-EYFP and Xrcc1-ECFP constructs were prepared as follows: total RNA was isolated from AA8 cells (RNeasy midi kit, Qiagen). First strand hamster Xrcc1 cDNA substrate was generated using dT-oligo and TaqMan Reverse Transcription Reagents (Applied Biosystem). PCR was carried out using AccuTaq LA DNA polymerase (Sigma–Aldrich), and oligonucleotides.

Xrcc1F-XhoI (5′-GAATTCCTCGAGGATGCCGGAGATCAGCCTCCGC-3′) and Xrcc1R-XmaI (5′-GAATTCCCCGGGCTCCGGCCTGTGGCACCACTC-3′). The PCR product was digested with XhoI/XmaI and cloned into XhoI/XmaI site of EYFP-N1 vector (Clontech). The deletion constructs were amplified using the following primer pairs:

For Xrcc1$_{1-325}$-ECFP: Xrcc1F-XhoI (5′-GAATTCCTCGAGGATGCCGGAGATCAGCCTCCGC-3′) and Xrcc1R2-XmaI (5′-GGATCCCCCGGGCCACCACACCCTGCAGAATC-3′).

For Xrcc1$_{1-180}$-ECFP: Xrcc1F-XhoI (5′-GAATTCCTCGAGGATGCCGGAGATCAGCCTCCGC-3′) and Xrcc1R3-EcoRI (5′-GGATCCGAATTCCTTTCACACGGAACTGGC-3′).

For Xrcc1$_{315-633}$-ECFP: Xrcc1F2-EcoRI (5′-GGATCCGAATTCGGAGCTGGGGAAGATTCTG-3′) and Xrcc1R-XmaI (5′-GAATTCCCCGGGCTCCGGCCTGTGGCACCACTC-3′).

PCR products were purified, digested with appropriate restriction enzymes, and cloned into the appropriate restriction sites of pECFP-N1. The primer sequences were derived from NCBI-AF034203.

2.3. Cell cultures

Cells were transfected with the different fusion constructs using calcium phosphate (Profection Promega) or Fugene 6 (Roche). HeLa and EM9 (Chinese hamster ovary cell line) cells stably expressing human or Chinese hamster XRCC1-EYFP or deletion constructs thereof, UNG2-EYFP and EYFP were prepared by transfection followed by cell sorting or cloning by dilution and prolonged culturing in selective media (DMEM (Gibco)) containing 10% fetal calf serum, gentamycin (0.1 mg/ml, Gibco), glutamine (1 mM), fungizone (2.5 μg/l) and geneticin (G418, 0.4 mg/ml, Invitrogen). For transient transfection the cells were examined after 16–24 h. Untransfected cells were cultured in the same medium without geneticin.

2.4. Proximity ligation assay (PLA) and immunofluorescence staining

PLA was performed to visualize UNG2/XRCC1 interactions in vivo. Briefly, in PLA oligonucleotide conjugated probe antibodies are directed against primary antibodies. Annealing of the probes occurs when the target proteins are in close proximity, which initiates the amplification of a reporter signal [31]. Cells were grown on coverslips overnight. Cells were washed once with phosphate-buffered saline (PBS) and fixed with 2% paraformaldehyde for 15 min on ice, washed once with PBS, permeabilized with methanol at −20 °C for 30 min, washed once with PBS–FCS (2% fetal calf serum in PBS) and blocked by incubation in PBS–FCS for 30 min prior to incubation with primary antibodies diluted in PBS–FCS (mouse monoclonal α-FLAG (1:1000) and rabbit polyclonal α-XRCC1 (1:250). PLA was performed as described by the manufacturer (Olink Biosciences) using the Duolink Detection Kit with PLA PLUS and MINUS probes. For immunohistochemistry, the cells were fixed, permeabilized and blocked and incubated with primary antibodies as above. Secondary antibodies used were Alexa fluor 532 goat-α-mouse IgG and Alexa Fluor 647 goat-α-rabbit IgG.

2.5. Confocal microscopy

Fluorescent images of live cells co-transfected with ECFP, EYFP and HcRed constructs (1 μm thickness) were produced using a Zeiss LSM 510 Meta laser scanning microscope equipped with a Plan-Apochromate 63×, 1.4 oil immersion objective. Three colour images were taken using three consecutive scans and the following settings: ECFP-excitation at λ = 458 nm, detection at λ = 470–500 nm, EYFP excitation at λ = 488 nm, at λ = 530–600 and HcRed excitation at λ = 543 nm, detection at λ > 615 nm. For immunofluorescence staining: excitation at λ = 543 nm and λ = 633 nm and detection at λ = 560–615 nm and λ > 650 nm respectively. PLA: excitation at λ = 543 nm and detection at λ = 560–615 nm, together with EYFP excitation at λ = 514 nm and detection at λ = 560–615 nm.

2.6. Cell synchronization

HeLa cells stably expressing XRCC1-EYFP or UNG2-EYFP or EYFP alone were synchronised by double thymidine block, which arrests the cells at the G1/S border [32]. The cells were released at mid-S-phase (3 h) [33]. Cell cycle phase was verified by flow cytometry. The DNA analysis of the cells was performed after methanol fixation (70%), RNase treatment (100 μg/ml in PBS at 37 °C for 30 min) and propidium iodide (PI) staining (50 μg/ml in PBS at 37 °C for 30 min) on a FACS Canto flow cytometer (BD-Life Science).

2.7. Formaldehyde crosslinking of proteins in intact cells and preparation of whole cell extracts

Cells were synchronised by double thymidine block [32] and analysed for cell phase by flow cytometry (BD Canto Flow) after methanol fixation, RNAse treatment and propidium iodide staining. The cell fractions enriched in S- and G1-phases were harvested and washed twice with cold PBS. $5–6 \times 10^6$ cells were resuspended in 10 ml PBS containing 0, 0.125 or 0.25% formaldehyde and incubated at 37 °C for 20 min. Crosslinking was stopped by adding glycine (final concentration 0.125 M) and further incubation at room temperature for 5 min. Cells (non-crosslinked and crosslinked) were collected by centrifugation and washed once in PBS, suspended in 8× packed cell volume (PCV) in buffer I (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 100 mM KCl, 0.2 mM EDTA, 20% (v/v) glycerol, 0.5% Nonidet P-40, 1 mM DTT and Complete protease inhibitor) containing 5 μl Omnicleave Endonuclease (200 U/μl Epicentre Technologies, WI) and sonicated. DNAse and RNase cocktail I (2 μl (200 U/μl) of Omnicleave Endonuclease, 1 μl Bensonase (250 U/ml, Novagene, Ge), 10 μl RNAse (10 mg/ml, Sigma–Aldrich), 1 μl DNAse (10 U/μl, Roche Inc.), and 1 μl micrococcal nuclease (100–300 U/mg, Sigma–Aldrich) per 30 mg cell extract) was added to the homogenate and incubated at room temperature for 1 h and dialyzed at 4 °C overnight in buffer II (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 100 mM KCl, 0.2 mM EDTA, 10% (v/v) glycerol, 1 mM DTT and Complete protease inhibitor). The extracts were cleared by centrifugation at 16,200 × *g* for 10 min and the supernatant was used for further analyses. For preparation of extracts from the chromatin enriched fraction, sonicated HeLa cells were pelleted at 16,200 × *g*. The pellet was resuspended in buffer II containing the DNAse and RNAse cocktail I and incubated at 37 °C for 1 h. The extract was cleared by centrifugation at 16,200 × *g* and the supernatant was used for immunoprecipitation with anti-EYFP coupled beads. The immunoprecipitation of all samples (formaldehyde crosslinked and non-crosslinked samples and the chromatin enriched samples) was carried out in 5 ml buffer II, and the beads were washed 5× with 1 ml buffer II prior to suspension in loading buffer. For the crosslinked samples the immunoprecipitates were heated at 65 °C for 10 min to release the proteins/protein complexes from the beads. Half of the released material (after removal of beads) was further incubated at 95 °C for 30 min to reverse the crosslinks. The proteins were separated on denaturing polyacrylamide gel and analysed by Western blot.

2.8. Pull-down assay and Far Western analysis

Cell extracts from EM9 cells expressing constructs of hamster Xrcc1-EYFP were incubated with anti-EYFP beads overnight. The beads were washed three times in 20 mM HEPES pH 7.5, 500 mM KCl, 2 mM DTT, 5 mM PMSF, Complete protease inhibitor and 1% Tween 20 in order to wash unrelated proteins off the fusion proteins. The beads were then washed once in 20 mM HEPES pH 7.5, 100 mM KCl, 2 mM DTT, 20 mg/ml BSA 1% Tween 20, 5 mM PMSF and Complete protease inhibitor and incubated with 0.2 μg/ml Δ93 UNG2 in the same buffer at 4 °C for 1 h under constant rotation. The beads were washed in 20 mM HEPES pH 7.5, 200 mM KCl, 2 mM DTT, 1% Tween 20, 5 mM PMSF and Complete protease inhibitor, and the pull-down material was analysed by Western blot. In the Far Western experiment, the immunoprecipitates were washed three times in 20 mM HEPES pH 7.5, 1 M KCl, 2 mM DTT, 5 mM PMSF and Complete protease inhibitor and 1% Tween 20, separated on Bis-Tris–HCl NuPAGE ready gels (4–12%) and transferred to PVDF membranes (Immobilon, Millipore). The membrane was incubated overnight in a buffer containing 10 mM Tris, pH 7.5, 0.5% BSA, 0.25% gelatin, 0.2% Triton X-100, 5 mM β-mercaptoethanol, and 150 mM NaCl. Fresh buffer containing 100 mM NaCl was added with 5 μg of recombinant Cy-3 labelled Δ93 UNG2 protein (Cy 3 Ab Labeling kit (PA33000), Amersham) and the membrane was incubated for a further 4–6 h, washed briefly at room temperature with buffer containing 10 mM Tris–HCl, pH 8, 0.05% Tween, and 100 mM NaCl. The membrane was dried and first analysed in a Typhoon Trio Imager (GE Healthcare), then stripped and probed with α-Xrcc1 antibody for Western analysis.

2.9. Immunoprecipitation and Western blot analysis

For immunoprecipitation, antibodies covalently linked to protein-A paramagnetic beads were added to the extracts in 5 ml buffer II and incubated at 4 °C overnight. The beads were collected from the solution, washed once in 1 ml of buffer II and thereafter three times in 10 mM Tris–HCl pH 7.5 and 50 mM KCl. The beads were used in BER assays (described below) or suspended in loading buffer, heated, separated on Bis-Tris–HCl NuPAGE ready gels and transferred to PVDF membranes. The membranes were blocked in 5% low fat dry milk in PBST (PBS with 0.1% Tween 20) and incubated with primary antibody in 1% dry milk at 4 °C overnight. Following incubation for 1 h with secondary antibody, membranes were treated with Chemiluminescence reagent (SuperSignal West Femto Maximum, PIERCE) and the proteins visualised in the Kodak Image Station 2000R.

2.10. BER assay

An illustration of DNA substrate and strategy for BER patch size analysis is shown in Fig. 3. To prepare the AP site substrate, we incubated uracil-containing DNA with purified catalytic domain of UNG [28]. The BER assay was carried out essentially as described [27,34]. The purified DNA was digested with appropriate restriction enzymes for BER analysis.

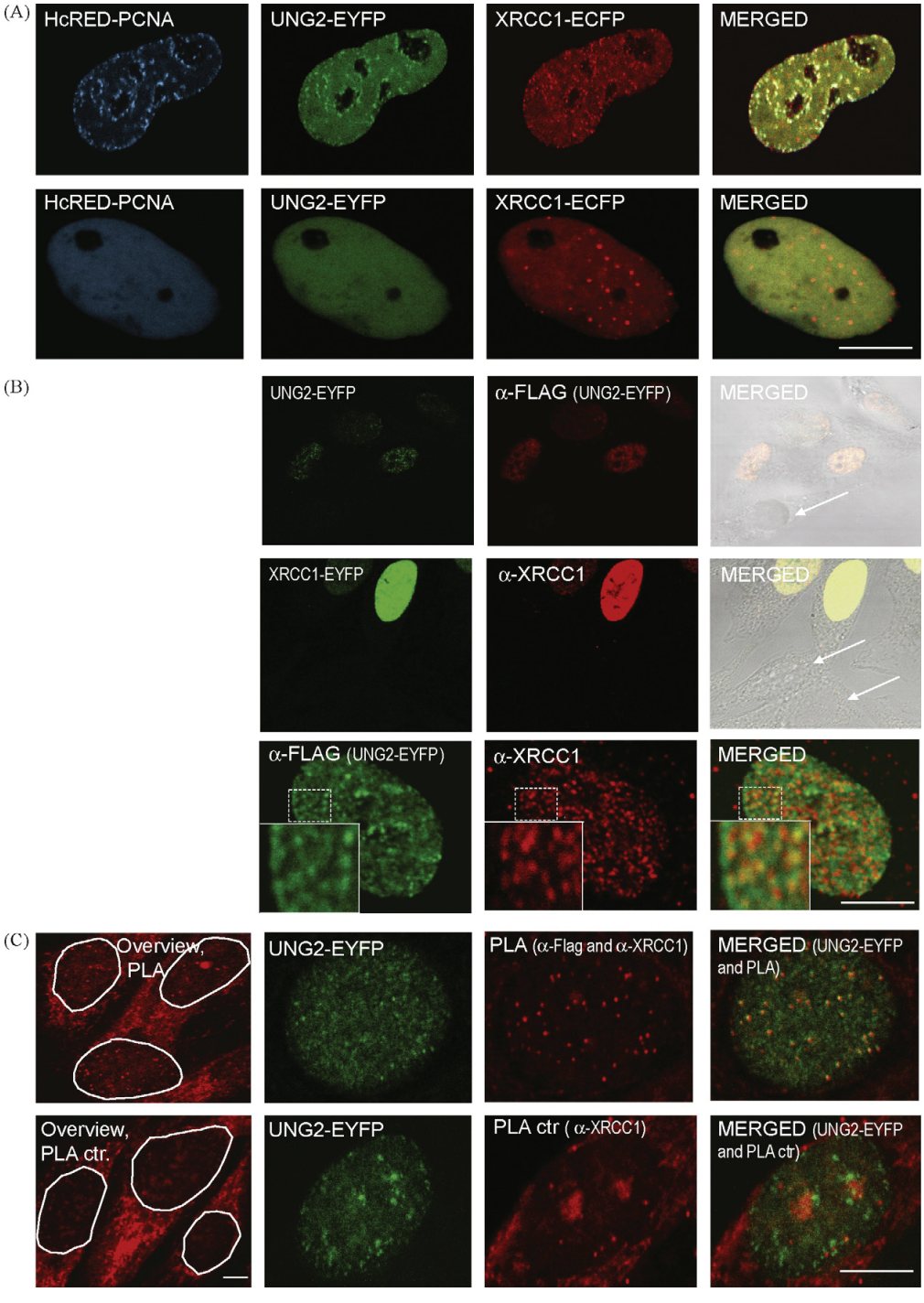
2.11. UDG activity assays

UDG activity was assayed according to previously described procedures [7,35]. Briefly, UDG activity was measured in 20 μl of assay mixture containing 20 mM Tris–HCl (pH 7.5), 50 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.5 mg/ml BSA, 1.8 μM [^{3}H]dUMP-containing calf thymus DNA (specific activity 0.5 mCi/μmol) and nuclear extracts. The mixture was incubated at 30 °C for 10 min and the amount of released uracil was measured by scintillation counting.

3. Results

3.1. XRCC1, UNG2 and PCNA co-localize in replication foci

Deamination of cytosine results in a U:G mismatch that can give rise to a C:G to T:A transition mutation if not repaired prior to replication. Previously, UNG2 was shown to localize in replication foci and to be responsible for immediate post-replicative removal of mis-incorporated uracil in DNA [8]. In view of the proposed role of XRCC1 in organising DNA repair ahead of replication [17], we first examined whether UNG2 and XRCC1 are simultaneously present in


(A)
HcRED-PCNA
UNG2-EYFP
XRCC1-ECFP
MERGED
HcRED-PCNA
UNG2-EYFP
XRCC1-ECFP
MERGED
(B)
UNG2-EYFP
α-FLAG (UNG2-EYFP)
MERGED
XRCC1-EYFP
α-XRCC1
MERGED
α-FLAG (UNG2-EYFP)
α-XRCC1
MERGED
(C)
Overview, PLA
UNG2-EYFP
PLA (α-Flag and α-XRCC1)
MERGED (UNG2-EYFP and PLA)
Overview, PLA ctr.
UNG2-EYFP
PLA ctr (α-XRCC1)
MERGED (UNG2-EYFP and PLA ctr)

replication foci during S-phase. Using HeLa cells transiently transfected with tagged proteins, we found that the PCNA foci contained both UNG2 and XRCC1 (Fig. 1A, white spots), while several XRCC1 foci in S-phase cells and all XRCC1 foci in non S-phase cells, contained neither UNG2 nor PCNA (Fig. 1A and B, red spots).

Next, we employed in situ Proximity Ligation Assay (PLA), a technique that combines dual recognition of target proteins using secondary antibodies with attached DNA strands as proximity probes, forming templates for rolling circle amplification for detecting protein interactions and/or proximity of proteins [31]. Two proteins can appear to co-localize in confocal microscopy even being up to 20-folds further apart [36] as compared to PLA [31]. So, PLA provides stronger indications for in situ protein–protein interactions. Expression of UNG2 is strictly cell cycle regulated, i.e. it increases in late G1, achieves maximal expression in mid-S-phase and becomes degraded in G2/M [33]. Thus, correct intra-cellular localization of UNG2 is likely to be tightly associated with its level of expression. We prepared cells stably expressing 3× FLAG-UNG2-EYFP (UNG2-EYFP) containing the native UNG2 promoter [7,37] and used this cell line for PLA analysis of UNG2 and XRCC1. For primary antibodies we used a monoclonal anti-FLAG antibody (α-FLAG) for UNG2 detection and a polyclonal antibody for XRCC1 detection (α-XRCC1). The specificity of these was verified in HeLa cells transfected with 3× FLAG-UNG2-EYFP (UNG2-EYFP) and Xrcc1 deficient (EM9) Chinese hamster ovary (CHO) cells (CHO-EM9) transfected with XRCC1-EYFP. Non-transfected cells, did not stain with these antibodies (Fig. 1B, see arrows). Similar to the co-localization seen in live cells (Fig. 1A), staining of stable UNG2-EYFP expressing cells with α-FLAG and α-XRCC1 antibodies identified nuclear foci containing both UNG2 and XRCC1 (Fig. 1B, lower row). By using both EYFP and Flag tagged UNG2, we could examine co-localization of PLA foci with UNG2 foci by detection of EYFP (UNG2-EYFP) as an extra quality control. When using these antibodies in the PLA system, we detected specific nuclear foci that co-localized with UNG2-EYFP foci (Fig. 1C, upper panel). In a control experiment, using PLA with only α-XRCC1, we detected a few foci (Fig. 1C, lower panel, PLA control), but less than 10% of these foci co-localized with UNG2-EYFP foci suggesting that they were unspecific. In contrast, more than 90% of the PLA foci using both α-FLAG and α-XRCC1 antibodies co-localized with UNG2-EYFP (Fig. 1C, upper panel, PLA). The overview images (Fig. 1C, left) show additional representative cells where the nuclei are outlined. In conclusion, the results of PLA assay show that UNG2 and XRCC1 are sufficiently close to each other in S-phase foci to suggest a direct interaction between the proteins.

3.2. UNG2 binds to the NLS region of XRCC1

One of the first indications for a direct interaction between XRCC1 and UNG2 arose when we attempted to purify human XRCC1 from *E. coli* extract. Bacterial *E. coli* Ung, which has high sequence homology (55.7% identity and 73.3% similarity when considering conserved residues) to the catalytic domain of human UNG2 [38], was readily co-purified with full length human XRCC1 (data not shown). Thus, we examined for an interaction of purified catalytic domain of human UNG2 with different constructs of *Cricetulus griseus* Xrcc1 fused with ECFP (illustrated in Fig. 2C), using a combination of immunoprecipitation, pull-down assay and Far Western. The constructs were expressed in CHO-EM9 ($Xrcc1^{-/-}$) cells to avoid interaction with endogenous Xrcc1 and to allow post-translational modification which may be important for protein interactions. Total cell extracts (DNase and RNase treated, see Section 2) from the plasmid transfected CHO cells were incubated with α-GFP (capable of capturing EGFP, ECFP and EYFP tags) coupled beads. The beads were washed extensively with high

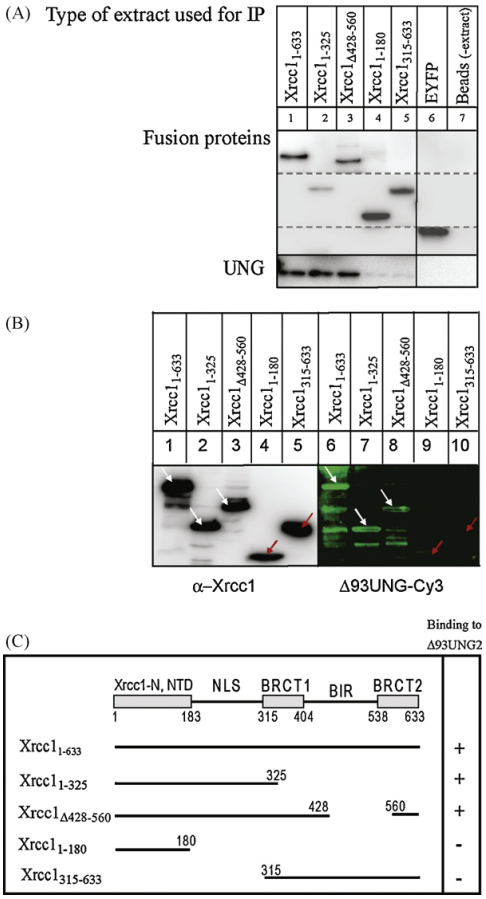


Fig. 2. Pull-down and Far Western analysis of Xrcc1 and UNG2 interaction. The indicated constructs of *Cricetulus griseus* Xrcc1 fused with ECFP were transiently expressed in Xrcc1 deficient Chinese hamster ovary cell line EM9. Cells expressing only EYFP were used as control. The fusion proteins were immunoprecipitated with paramagnetic beads covalently coupled with α-GFP. (A) The immunoprecipitates were incubated with the catalytic domain of UNG2 (Δ93UNG2). The beads were suspended in loading buffer and subjected to Western analysis. Lane 7, the beads were only incubated with recombinant UNG. The membrane was incubated with α-GFP antibody (upper panel) and α-UNG antibody (lower panel). (B) Far Western analysis. The immunoprecipitates were separated on gel and transferred to a PDF membrane and refolded. The membrane was first incubated with Cy-3 labelled Δ93UNG2 then stripped and probed with α-XRCC1. White arrows shows binding of Δ93UNG2 to XRCC1 fusion construct, while red arrows indicate no binding. (C) Schematic overview of the XRCC1 deletion constructs applied and their ability to bind to recombinant Δ93UNG2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Upper row: HeLa cells expressing 3× FLAG-UNG2-EYFP (UNG2-EYFP) were fixed and incubated with a monoclonal α-FLAG antibody and a polyclonal α-XRCC1 antibody followed by detection of PLA foci using oligonucleotide conjugated probe antibodies directed against the primary antibodies. Annealing of the probes occurs when the target proteins are in close proximity, which initiates the amplification of a reporter signal and appears as foci (PLA, α-FLAG and α-XRCC1). An overview of several cells after PLA (overview, PLA) (far left, the nuclei are enclosed) and a merged image of PLA and UNG2-EYFP are shown (merged) (far right). Lower row: same as upper row but the cells were not stained with the α-FLAG antibody (PLA ctr). Bar, 5 μm.

salt wash buffer and then incubated with the catalytic domain of UNG2 (Δ93 UNG2) [28] for pull-down analysis or separated by gel electrophoresis and transferred to a membrane for Far Western analysis.

Western blot analysis of the pull-down material showed that the fusion proteins containing the NLS region of Xrcc1 as well as full length Xrcc1-ECFP were able to pull-down Δ93 UNG2 (Fig. 2A). This result suggests that UNG2 directly interacts with Xrcc1 at the NLS region of Xrcc1.

To further verify a direct interaction between UNG2 and Xrcc1, and exclude the possibility that this binding is mediated via a common binding protein such as PCNA, we performed Far Western analysis incubating the membrane containing the different fusions proteins immunoprecipitated from transiently transfected CHO-EM9 cells with Cy-3 labelled Δ93 UNG2. Fig. 2B shows specific Cy-3 bands corresponding in size to full length Xrcc1, $Xrcc1_{1-325}$ and $Xrcc1_{\Delta 428-560}$ fusion proteins (lanes 6–8 and 1–3 respectively, white arrows). No specific bands for UNG2 can be detected for the $Xrcc1_{1-180}$ and $Xrcc1_{315-633}$ fusion proteins (lanes 9–10, and 4–5, respectively, red arrows). Some of the degraded fusion proteins in lanes 1–3 were also able to interact with Cy-3 labelled UNG2 (lanes 6–8). In summary, these results clearly demonstrate a direct interaction between Xrcc1 and UNG2. Based on the results shown in Figs. 2–5 (see also below), only a sub-fraction of Xrcc1 binds UNG2, possibly mediated by a post-translational modification on Xrcc1.

3.3. XRCC1 immunoprecipitates carry out UNG specific uracil-BER

To examine whether XRCC1-EYFP immunoprecipitates were proficient in uracil-BER we carried out functional analysis of the immunoprecipitates using a closed circular DNA containing uracil at a defined position (Fig. 3, left panel). Compared to the robust capacity for complete BER of AP sites (Fig. 3, right panel, lanes 7–9), we detected a low, but significant, level of uracil-BER activity in the immunoprecipitates (lanes 1 and 4). The latter activity was completely inhibited by neutralising α-UNG [28] (lanes 2 and 5) or Ugi [39] (lanes 3 and 6). These results indicate that XRCC1-EYFP immunoprecipitates contain functional repair complexes with a capacity for UNG2 specific uracil-BER.

3.4. UNG2 and XRCC1 are part of a common complex as well as distinct repair complexes in S-phase cells

UNG2 co-localized with XRCC1 in replication foci (Fig. 1A), thus we next examined whether XRCC1 and UNG2 are present in a common or distinct replication associated complexes. For this we used 3× FLAG-UNG2-EYFP expressing cells, cells expressing XRCC1-EYFP and cells only expressing EYFP. We employed in vivo formaldehyde crosslinking and immunoprecipitation with antibodies against the EYFP tag to analyze the protein composition of the immunoprecipitates. Whole cell extracts were prepared from formaldehyde crosslinked and non-crosslinked cells enriched at the G1/S-boarder and mid-S-phase (double thymidine block synchronization). Immunoprecipitates from EYFP expressing cells were used as the control. Importantly, the experiments were carried out under identical conditions with the same antibody.

Formaldehyde crosslinking is a sensitive method that needs to be optimized to avoid excessive crosslinking. To examine the extent of crosslinking, we carried out BER analysis of XRCC1-EYFP immunoprecipitates from crosslinked cell extracts. Since the immunoprecipitates showed detectable uracil and AP site BER activity (Fig. 4A), a considerable number of proteins in the immunoprecipitates were functional, illustrating that the crosslinking was mild under our experimental conditions.

For Western analysis, the immunoprecipitates were suspended in loading buffer and heated at 65 °C for 10 min to release proteins from the beads (most crosslinks still present). Half of the eluted material (after removal of the beads) was further heated at 95 °C for 30 min to reverse the crosslinks. The results show that close to equal amounts of XRCC1-EYFP and UNG2-EYFP were immunoprecipitated from the crosslinked and non-crosslinked samples (Fig. 4B, compare fusion proteins at different formaldehyde concentrations). This further supports that under our experimental conditions, the crosslinking was mild, as excessive crosslinking would have likely resulted in a considerable reduction in the amount of immunoprecipitated fusion protein from the samples due to loss of available epitopes. We detected high molecular weight proteins or protein complexes (>200 kDa) in the immunoprecipitates from the crosslinked extracts containing XRCC1 and POLδ (Fig. 4B, first and third column, marked with red asterisk). Much less of these bands were detected after reversal of the crosslinks by heating at 95 °C, whereas the bands for XRCC1 and POLδ became correspondingly more intense (Fig. 4B, second and fourth columns). Notably, the high molecular weight complexes appeared to be more pronounced in the XRCC1-EYFP immunoprecipitates from the mid-S-phase extracts compared to the G1/S extracts, suggesting an increase in the amount of large XRCC1-EYFP complexes in S-phase. In agreement with the confocal images, in which we identified co-localization of XRCC1 and UNG2 only in replication foci (Fig. 1A), we detected more UNG2 and PCNA in

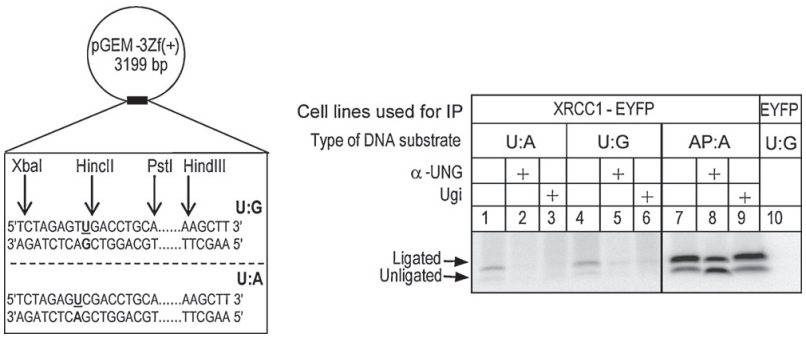


Fig. 3. BER analysis of XRCC1-EYFP immunoprecipitates from whole cell extract. Left panel: schematic illustration of U:G and U:A DNA substrates for BER analysis. AP site DNA was prepared by incubation of uracil-containing DNA with recombinant UNG. Right panel: lanes 1–6, uracil-BER analysis of XRCC1-EYFP complexes in the absence or presence of UNG2 neutralising antibodies (α-UNG2) or Ugi. Lanes 7–9, analysis of AP site BER. Lane 10 shows BER analysis of the control immunoprecipitates from EYFP expressing cells. The repair reaction was carried out at 32 °C for 15 min. The repaired DNA was digested with XbaI/HindIII to get information about the ligated and unligated repair products as shown.

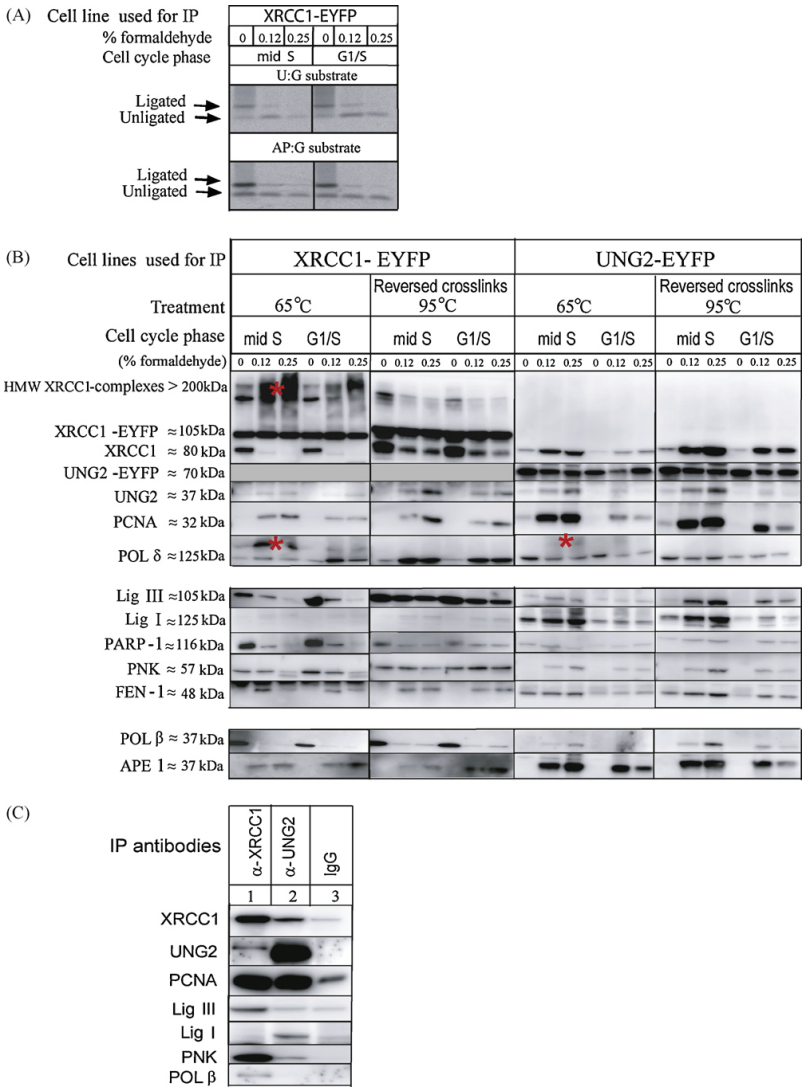


Fig. 4. Comparative Western blot analysis of XRCC1-EYFP and UNG2-EYFP immunoprecipitates from extracts of mid-S- and G1/S-phase enriched cells. (A) BER activity of immunoprecipitated XRCC1-EYFP from crosslinked and non-crosslinked cell extracts. BER was carried out at 32 °C for 15 min. The repaired DNA was digested with XbaI and HindIII to get information about the ligated and unligated repair products. (B) Western analysis of immunoprecipitates from formaldehyde crosslinked and non-crosslinked XRCC1-EYFP and UNG2-EYFP expressing HeLa cell extracts. Cells were treated with formaldehyde (0, 0.125 or 0.25%) prior to whole cell extract preparation. Western blot analysis of the immunoprecipitates before (65 °C) and after (95 °C) reversal of the crosslinks is shown. (C) Western analysis of immunoprecipitates from insoluble chromatin-bound fraction of freely cycling HeLa cells using anti-XRCC1 (lane 1) or anti-UNG (lane 2) antibodies. One representative gel from three experiments is shown.

immunoprecipitates from mid-S-phase XRCC1-EYFP extracts, as well as higher amounts of XRCC1 and PCNA in immunoprecipitates from mid-S-phase UNG2-EYFP extracts, as compared to immunoprecipitates from G1/S boarder cell extracts (Fig. 4B, second and fourth columns). Western analysis of immunoprecipitates from the control EYFP expressing cells was negative for XRCC1, UNG2, PCNA, POLδ, Lig III, Lig I, PARP-1, PNK and APE-1 (data not shown), demonstrating that non-specific binding to beads and excessive non-specific crosslinking of proteins in vivo was not a problem under the conditions employed. This observation is in agreement with a recent study showing that immunoprecipitation of tagged proteins with magnetic beads display a low level of unspecific binding to the immunoprecipitates compared to several available methods [40].

The results thus far suggested that UNG2 and XRCC1 are likely part of a common multiprotein complex in replication foci. However, the overall composition of XRCC1-EYFP and UNG2-EYFP associated proteins varied significantly with respect to the amount of Lig III, Lig I, PARP-1 and PNK in the immunoprecipitates (Fig. 4B, mid-panel). For example, the XRCC1 partner, Lig III [16], was readily detected in the XRCC1-EYFP immunoprecipitates both without and after crosslinking (Fig. 4B, second column), whereas in the UNG2-EYFP immunoprecipitates, the amount of Lig III followed the amounts of XRCC1, i.e. more was detected

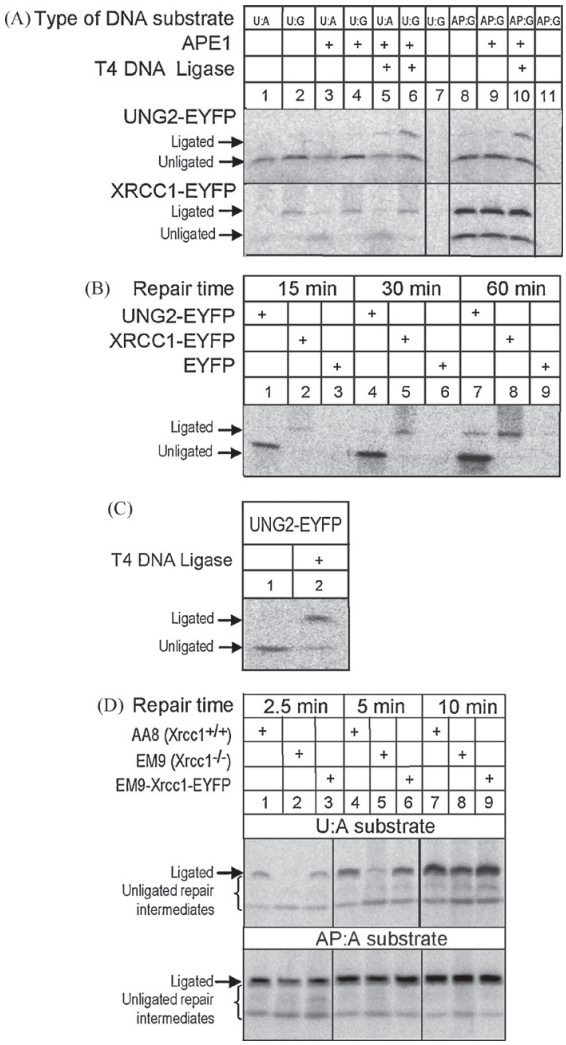


Fig. 5. BER analysis of UNG2-EYFP and XRCC1-EYFP immunoprecipitates from mid-S-phase enriched cells and BER analysis of cell extracts in the absence or presence of Xrcc1. (A) BER activity of the immunoprecipitated UNG2-EYFP and XRCC1-EYFP complexes was analyzed using U:A, U:G and AP:G DNA substrates with or without additional recombinant APE1 and T4 DNA ligase at 32 °C for 15 min. Lanes 7 and 11 show repair activity of the immunoprecipitates from EYFP expressing cell extracts as control. (B) Time-course BER analysis of the immunoprecipitated UNG2-EYFP and XRCC1-EYFP complexes using U:G substrate. (C) End-trimming and DNA ligase activity of UNG2-EYFP immunoprecipitates. After 1 h incubation, half of the repaired DNA was further incubated with T4 DNA ligase (lane 2). (D) Time-course BER analysis of extracts from AA8 ($Xrcc1^{+/+}$), EM9 ($Xrcc1^{-/-}$) and EM9 cells expressing Xrcc1-EYFP (EM9-Xrcc1-EYFP) on a U:A and AP:A substrate. All DNA samples were digested with XbaI and HindIII to get information about DNA ligase and end-trimming activity of the immunoprecipitates.

in mid-S-phase extracts (Fig. 4B, fourth column). Lig I was barley visible in any XRCC1-EYPF immunoprecipitates, while it was readily detected in all UNG2-EYFP immunoprecipitates, with more in the mid-S-phase immunoprecipitates (Fig. 4B, mid-panel, second and fourth columns). Thus, XRCC1 and UNG2 seem to be part of separate complexes, as well as a common complex, during S-phase.

Interestingly, the amounts of PARP-1, POLβ and Lig III were higher in the immunoprecipitates from non-crosslinked XRCC1-EYFP extracts than from the crosslinked extracts (Fig. 4B, mid- and lower panel). This may be due to protein interactions that take place in the extracts before or during immunoprecipitation, which may otherwise occur at a lower extent in situ. On the other hand, APE1 was only detected in the immunoprecipitates from the crosslinked cells (Fig. 4B, lower panel), suggesting that APE1 associates with XRCC1 and UNG2 with low affinity, so most of it may fall off during immunoprecipitation. This seemingly agrees with the disparate reports on the interaction of APE1 with XRCC1 at different experimental conditions [14,17]. However, immunoprecipitated XRCC1-EYFP complexes clearly contained AP-endonuclease activity (Figs. 3 and 4A), indicating the presence of APE1, even though it was not detected by Western analysis from non-crosslinked extract.

3.5. XRCC1 and UNG2 are part of a common complex in chromatin

Because the confocal studies showed that XRCC1 and UNG2 co-localize in replication foci and Western analysis suggested that these two proteins are present in a common, higher order complex during S-phase, we fractionated HeLa cells and carried out immunoprecipitation of a chromatin enriched fraction, which likely contains replication associated complexes. We used antibodies against endogenous UNG2 and XRCC1 for immunoprecipitation. Fig. 4C shows that similar to the results from the crosslinked samples, Lig III and PNK were readily detected in the XRCC1 immunoprecipitates, while Lig I was only detected in UNG2 immunoprecipitates. A low amount of UNG2 was detected in the XRCC1 immunoprecipitate and a low amount of XRCC1 was detected in the UNG2 immunoprecipitate. These data support the notion that XRCC1 and UNG2 are part of distinct as well as shared complexes, where only a fraction of XRCC1-associated complexes contain UNG2 and vice versa. These results also show that the overall organisation of protein–protein interactions within immunoprecipitates of fusion proteins using tag specific antibodies is largely identical to those detected in immunoprecipitates of corresponding endogenous XRCC1 or UNG2 proteins.

3.6. XRCC1 complexes immunoprecipitated from S-phase cells perform complete uracil and AP site repair

Next, we carried out comparative BER analysis of the immunoprecipitated XRCC1-EYFP and UNG2-EYFP from mid-S-phase enriched cells for repair of uracil and AP site in DNA (U:A and U:G). We found that UNG2-EYFP immunoprecipitates were unable to efficiently generate the final ligated product with any of the substrates (Fig. 5A, upper panel, lanes 1, 2 and 8), whereas complete BER was carried out by the XRCC1-EYFP immunoprecipitates at the same time (15 min) (Fig. 5A, lower panel, same lanes). To examine whether the levels of APE1 or a DNA ligase were responsible for the observed deficiency in repair activity of the UNG2-EYFP immunoprecipitates, we carried out BER analysis in the presence of supplemented APE1 or T4 DNA ligase. Addition of APE1 had no detectable effect on the amount of incorporated nucleotide during BER, indicating that sufficient AP-endonuclease activity was present in the UNG2-EYFP immunoprecipitates (Fig. 5A, upper panel, lanes 3, 4 and 9). In contrast, addition of T4 DNA ligase increased the amount of ligated products significantly (Fig. 5A, upper panel, lanes 5, 6 and 10), indicating that the ligation step was slow despite the presence of Lig I in similar immunoprecipitates (Fig. 4B and C). On the other hand, the XRCC1-EYFP immunoprecipitates performed complete uracil and AP site BER, and addition

of recombinant APE1 and T4 DNA ligase had no detectable effect on total BER (Fig. 5A, lower panel, lanes 3–6 and 9 and 10).

Next, we carried out time-course kinetic analysis of uracil-BER by the different immunoprecipitates and found that the amount of ligated products by UNG2 immunoprecipitates increased with prolonged incubation, but the unligated products were still more abundant (Fig. 5B, lane 7). The XRCC1-EYFP immunoprecipitates on the other hand, appeared to complete the repair pathway rapidly, and no unligated repair intermediates could be detected at any time point (Fig. 5B, lanes 2, 5 and 8). To examine whether the low level of ligated products in the UNG2 immunoprecipitates was due to unligatable DNA repair intermediates, we carried out uracil-BER of UNG2 immunoprecipitates for 60 min, and then incubated half of the purified DNA with excess T4 DNA ligase (see Section 2), which is expected to seal all ligatable ends [41]. DNA was then analysed for ligated and unligated repair products. The amount of ligated products increased, similar to what was found after addition of T4 DNA ligase in Fig. 5A (lane 10). However approximately 45% of the DNA was still unligated (Fig. 5C). This result indicates that also DNA end-trimming was less efficient in UNG2 immunoprecipitates as compared to XRCC1 immunoprecipitates. In summary, we have identified two functionally different S-phase associated BER complexes based on immunoprecipitation of UNG2 and XRCC1.

3.7. XRCC1 affects the rate of uracil-BER

We next examined if absence of XRCC1 could have any effect on repair of uracil by cell extracts. We examined the repair efficiency of cell extracts from wild type (AA8) and Xrcc1 deficient (EM9) cell lines, as well as an EM9 cell line expressing *Cricetulus griseus* Xrcc1-EYFP fusion protein. Xrcc1-EYFP was functional because it restored DNA repair capacity of EM9 cells following hydrogen peroxide challenge as assessed by the Comet assay (data not shown). We could not include the Xrcc1 deletion mutants used for pull-down experiments in this experiment, because we failed to establish EM9 cells stably expressing the respective constructs.

In order to distinguish between UNG2 and the other uracil-DNA glycosylases (SMUG1, TDG and MBD4) present in the cell extract, and thereby to focus on a biological role of the Xrcc1-UNG2 interaction, we used a U:A containing substrate which is strictly processed by UNG2 [7,42]. The U:G substrate, arising following deamination of cytosine, on the other hand, is efficiently corrected by SMUG1, TDG, MBD4 as well as UNG2 [43]. BER analysis for repair of U:A showed that after 5 min the level of BER was considerably lower in the Xrcc1 deficient extract, as compared to extracts from the wild type or Xrcc1-EYFP expressing cells (Fig. 5D, upper panel, lanes 4–6). However, this was not seen for AP site repair under identical conditions (Fig. 5D, lower panel, lanes 4–6), indicating that the slower uracil-BER in the absence of Xrcc1 was unlikely due to overall reduced stability of POLβ or Lig III in these cells [44,45]. To rule out that the observed differences in U:A BER were related to varying ability of the extracts for uracil excision (UDG) from the U:A context, we performed a standard UDG activity assay [36]. We found that extracts from the Xrcc1 deficient cells had 20% higher UDG activity compared to the wild type cell extract, indicating that the slower U:A BER in the Xrcc1 deficient cell extracts was not simply related to UDG capacity of the extract, but more likely due to a role for XRCC1 in coordinating the repair of uracil.

In summary, we have identified two functionally different S-phase associated BER complexes based on immunoprecipitation of UNG2 and XRCC1. A sub-fraction of the XRCC1 complex also contained UNG2, probably mediated through a direct binding between the NLS region of XRCC1 and catalytic domain of UNG2. We have presented data indicating reduced repair of uracil in cell extracts from Xrcc1$^{-/-}$ cells as compared to wild type cells and cells reconstituted with Xrcc1-EYFP, suggesting a functional implication of the XRCC1-UNG2 interaction in vivo.

4. Discussion

Both UNG2 and XRCC1 interact with PCNA in replication foci [8,17,46], thus we wanted to examine whether XRCC1 and UNG2 directly interact and are part of a complex involved in repair of uracil. Here we show that XRCC1 and UNG2 co-localize at sites of DNA replication and likely physically interact with each other at these sites. We show a direct interaction between the catalytic domain of UNG2 and the NLS region of XRCC1. In agreement with this, the results of BER assay analysis showed that XRCC1-EYFP immunoprecipitates from S-phase cell extracts contained UNG2-specific repair activity. Furthermore, we show that Xrcc1 deficient cells have reduced initial UNG2-specific uracil (U:A) repair.

To compare the composition of the XRCC1 and UNG2 associated complexes, we analysed complexes isolated from G1/S and mid-S-phase cells. We designed our study in a way that allowed us to immunoprecipitate target proteins from different cell lines using the same tag-specific antibody, thus avoiding variations due to different degrees of antibody specificity. Moreover, this strategy permitted us to avoid the potential problem of competition between antibody and proteins for binding to the same site on the target protein. Stringent washing of immunoprecipitates can reduce the level of non-specific protein–antibody interactions, yet some weakly bound, but physiologically relevant, proteins may be disrupted. To circumvent this problem, we included in vivo formaldehyde crosslinking of proteins, enabling us to observe proteins not detectable in non-crosslinked samples under otherwise identical conditions. Moreover, formaldehyde crosslinking of proteins in intact cells stabilizes protein–protein interactions as they take place "in situ". The results supported the presence of UNG2 in an S-phase XRCC1 complex. Furthermore, the data supported specific interaction of Lig III, POLβ and PNK with XRCC1 and specific interaction of Lig I with UNG2, indicating the presence of separate UNG2- and XRCC1-associated complexes.

Two important functional differences were detected between the immunoprecipitated XRCC1 and UNG2 complexes: (1) XRCC1 complexes carried out fast and complete BER (including ligation) and (2) UNG2 complexes did not perform efficient ligation. This was unexpected because the immunoprecipitates contained FEN-1 and Lig I. However, this could be explained by differences in organisation of the complexes as discussed below and illustrated in Fig. 6.

Because UNG2 and XRCC1 only co-localize in replication foci during S-phase, and not in other non-S-phase XRCC1 foci, it is tempting to speculate that the XRCC1-UNG2 complex have a specific repair function during replication. UNG2 is the only UDG known to be localized in replication foci [43]. We have previously suggested the presence of a pre-replicative XRCC1-SSBR complex [17], and based on the data presented in this study, we now extend this model to include pre-replicative BER. In this model, pre-replicative XRCC1-UNG2 complexes may facilitate efficient repair of mutagenic U:G mismatches ahead of replication. We also suggest that pre-replicative XRCC1-BER complexes may be important for the repair of other mutagenic base lesions such as AP sites and 3-methyladenine (3meA). 3meA is removed from DNA by the MPG DNA glycosylase, which interacts with XRCC1 [22] as well as PCNA [24]. Lack of XRCC1 may therefore at least in part explain the high sensitivity of XRCC1 deficient cells to MMS [47].

Previous data have shown that UNG2 rapidly removes misincorporated dUMP during replication [8]. During replication, DNA synthesis on the leading and lagging strands may involve different forms of protein interactions and complexes. Thus, whereas continuous DNA synthesis on the leading strand is rapid and proces-

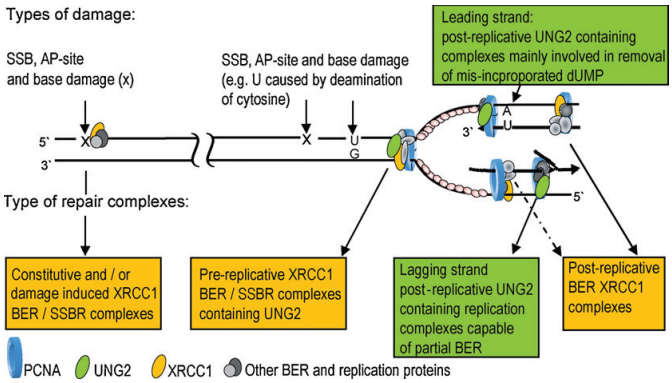


Fig. 6. A model for repair of uracil in DNA by XRCC1 and UNG2 complexes. UNG2 is linked to replication through its interaction with XRCC1 and PCNA, both of which interact with a number of proteins involved in DNA replication and repair. We suggest a model for how these interactions organize efficient pre-replicative repair of U:G mismatch and post-replicative repair of mis-incorporated uracil (U:A) in DNA, during replication.

sive, and does not normally involve FEN-I and Lig I, DNA replication on the lagging strand requires rapid engagement of proteins also involved in long patch BER, such as POLδ, FEN1 and Lig I. Since the majority of the UNG2 associated complexes isolated from S-phase were inefficient in the ligation step of BER, post-replicative repair of uracil [8] may be organized into different forms on the leading and lagging strands. Thus, in order to retain the continuity of DNA replication on the leading strand, removal of uracil from DNA by UNG2 [8] may not be coupled to the later repair steps, and the following AP site may be rapidly repaired by post-replicative XRCC1 complexes. However, on the lagging strand, UNG2 may be part of a replication/repair complex that carries out uracil removal followed by AP site incision and repair synthesis probably in the form of long patch BER. Our proposed model for replication associated BER is shown in Fig. 6.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We would like to thank Ruth Haaland Krokan and Nina-Beate Liabakk for technical assistance. This work is supported by The Research Council of Norway, The National Programme for Research in Functional Genomics in Norway (FUGE) in The Research Council of Norway, The Cancer Fund at St. Olav's Hospital, Trondheim, Norway, The Norwegian Cancer Society, The Svanhild and Arne Must Fund for Medical Research, Norway, Integrated Project on DNA Repair Supported by European Union and the Intramural Research Program of the NIH, National Institute on Aging, USA.

References

[1] T. Lindahl, Instability and decay of the primary structure of DNA, Nature 362 (1993) 709–715.
[2] A. Verri, P. Mazzarello, G. Biamonti, S. Spadari, F. Focher, The specific binding of nuclear protein(s) to the cAMP responsive element (CRE) sequence (TGACGTCA) is reduced by the misincorporation of U and increased by the deamination of C, Nucleic Acids Res. 18 (1990) 5775–5780.
[3] A. Viswanathan, H.J. You, P.W. Doetsch, Phenotypic change caused by transcriptional bypass of uracil in nondividing cells, Science 284 (1999) 159–162.
[4] M. Akbari, J. Pena-Diaz, S. Andersen, N.B. Liabakk, M. Otterlei, H.E. Krokan, Extracts of proliferating and non-proliferating human cells display different base excision pathways and repair fidelity, DNA Repair (Amst.) 8 (2009) 834–843.
[5] R.J. Sanderson, D.W. Mosbaugh, Fidelity and mutational specificity of uracil-initiated base excision DNA repair synthesis in human glioblastoma cell extracts, J. Biol. Chem. 273 (1998) 24822–24831.
[6] H.E. Krokan, F. Drablos, G. Slupphaug, Uracil in DNA—occurrence, consequences and repair, Oncogene 21 (2002) 8935–8948.
[7] B. Kavli, O. Sundheim, M. Akbari, M. Otterlei, H. Nilsen, F. Skorpen, P.A. Aas, L. Hagen, H.E. Krokan, G. Slupphaug, hUNG2 is the major repair enzyme for removal of uracil from U:A matches, U:G mismatches, and U in single-stranded DNA, with hSMUG1 as a broad specificity backup, J. Biol. Chem. 277 (2002) 39926–39936.
[8] M. Otterlei, E. Warbrick, T.A. Nagelhus, T. Haug, G. Slupphaug, M. Akbari, P.A. Aas, K. Steinsbekk, O. Bakke, H.E. Krokan, Post-replicative base excision repair in replication foci, EMBO J. 18 (1999) 3834–3844.
[9] K. Imai, G. Slupphaug, W.I. Lee, P. Revy, S. Nonoyama, N. Catalan, L. Yel, M. Forveille, B. Kavli, H.E. Krokan, H.D. Ochs, A. Fischer, A. Durandy, Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination, Nat. Immunol. 4 (2003) 1023–1028.
[10] C. Rada, G.T. Williams, H. Nilsen, D.E. Barnes, T. Lindahl, M.S. Neuberger, Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice, Curr. Biol. 12 (2002) 1748–1755.
[11] Q. An, P. Robins, T. Lindahl, D.E. Barnes, C→T mutagenesis and gamma-radiation sensitivity due to deficiency in the Smug1 and Ung DNA glycosylases, EMBO J. 24 (2005) 2205–2213.
[12] J. Fan, D.M. Wilson 3rd, Protein–protein interactions and posttranslational modifications in mammalian base excision repair, Free Radic. Biol. Med. 38 (2005) 1121–1138.
[13] L.H. Thompson, K.W. Brookman, N.J. Jones, S.A. Allen, A.V. Carrano, Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange, Mol. Cell Biol. 10 (1990) 6160–6171.
[14] A.E. Vidal, S. Boiteux, I.D. Hickson, J.P. Radicella, XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein–protein interactions, EMBO J. 20 (2001) 6530–6539.
[15] K.W. Caldecott, S. Aoufouchi, P. Johnson, S. Shall, 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 Res. 24 (1996) 4387–4394.
[16] K.W. Caldecott, C.K. McKeown, J.D. Tucker, S. Ljungquist, L.H. Thompson, An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III, Mol. Cell Biol. 14 (1994) 68–76.
[17] J. Fan, M. Otterlei, H.K. Wong, A.E. Tomkinson, D.M. Wilson 3rd, XRCC1 co-localizes and physically interacts with PCNA, Nucleic Acids Res. 32 (2004) 2193–2201.
[18] M. Masson, C. Niedergang, V. Schreiber, S. Muller, J. Menissier-de Murcia, G. de Murcia, XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage, Mol. Cell Biol. 18 (1998) 3563–3571.
[19] V. Schreiber, J.C. Ame, P. Dolle, I. Schultz, B. Rinaldi, V. Fraulob, J. Menissier-de Murcia, G. de Murcia, Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1, J. Biol. Chem. 277 (2002) 23028–23036.
[20] C.J. Whitehouse, R.M. Taylor, A. Thistlethwaite, H. Zhang, F. Karimi-Busheri, D.D. Lasko, M. Weinfeld, K.W. Caldecott, XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair, Cell 104 (2001) 107–117.
[21] S. Marsin, A.E. Vidal, M. Sossou, J. Menissier-de Murcia, F. Le Page, S. Boiteux, G. de Murcia, J.P. Radicella, Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1, J. Biol. Chem. 278 (2003) 44068–44074.
[22] A. Campalans, S. Marsin, Y. Nakabeppu, R. O'Connor, T.S. Boiteux, J.P. Radicella, XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair, DNA Repair (Amst.) 4 (2005) 826–835.

[23] I. Boldogh, D. Milligan, M.S. Lee, H. Bassett, R.S. Lloyd, A.K. McCullough, hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence suggesting replication-coupled repair of adenine:8-oxoguanine mispairs, Nucleic Acids Res. 29 (2001) 2802–2809.
[24] L. Xia, L. Zheng, H.W. Lee, S.E. Bates, L. Federico, B. Shen, T.R. O'Connor, Human 3-methyladenine-DNA glycosylase: effect of sequence context on excision, association with PCNA, and stimulation by AP endonuclease, J. Mol. Biol. 346 (2005) 1259–1274.
[25] G.L. Moldovan, B. Pfander, S. Jentsch, PCNA, the maestro of the replication fork, Cell 129 (2007) 665–679.
[26] A. Parker, Y. Gu, W. Mahoney, S.H. Lee, K.K. Singh, A.L. Lu, Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair, J. Biol. Chem. 276 (2001) 5547–5555.
[27] M. Akbari, M. Otterlei, J. Pena-Diaz, P.A. Aas, B. Kavli, N.B. Liabakk, L. Hagen, K. Imai, A. Durandy, G. Slupphaug, H.E. Krokan, Repair of U/G and U/A in DNA by UNG2-associated repair complexes takes place predominantly by short-patch repair both in proliferating and growth-arrested cells, Nucleic Acids Res. 32 (2004) 5486–5498.
[28] G. Slupphaug, I. Eftedal, B. Kavli, S. Bharati, N.M. Helle, T. Haug, D.W. Levine, H.E. Krokan, Properties of a recombinant human uracil-DNA glycosylase from the UNG gene and evidence that UNG encodes the major uracil-DNA glycosylase, Biochemistry 34 (1995) 128–138.
[29] P.A. Aas, M. Otterlei, P.O. Falnes, C.B. Vagbo, F. Skorpen, M. Akbari, O. Sundheim, M. Bjoras, G. Slupphaug, E. Seeberg, H.E. Krokan, Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA, Nature 421 (2003) 859–863.
[30] P.L. Opresko, M. Otterlei, J. Graakjaer, P. Bruheim, L. Dawut, S. Kolvraa, A. May, M.M. Seidman, V.A. Bohr, The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2, Mol. Cell 14 (2004) 763–774.
[31] O. Soderberg, M. Gullberg, M. Jarvius, K. Ridderstrale, K.J. Leuchowius, J. Jarvius, K. Wester, P. Hydbring, F. Bahram, L.G. Larsson, U. Landegren, Direct observation of individual endogenous protein complexes in situ by proximity ligation, Nat. Methods 3 (2006) 995–1000.
[32] R.T. Richardson, I.N. Batova, E.E. Widgren, L.X. Zheng, M. Whitfield, W.F. Marzluff, M.G. O'Rand, Characterization of the histone H1-binding protein, NASP, as a cell cycle-regulated somatic protein, J. Biol. Chem. 275 (2000) 30378–30386.
[33] L. Hagen, B. Kavli, M.M. Sousa, K. Torseth, N.B. Liabakk, O. Sundheim, J. Pena-Diaz, M. Otterlei, O. Horning, O.N. Jensen, H.E. Krokan, G. Slupphaug, Cell cycle-specific UNG2 phosphorylations regulate protein turnover, activity and association with RPA, EMBO J. 27 (2008) 51–61.
[34] G. Frosina, E. Cappelli, P. Fortini, E. Dogliotti, In vitro base excision repair assay using mammalian cell extracts, Methods Mol. Biol. 113 (1999) 301–315.
[35] H. Krokan, C.U. Wittwer, Uracil DNa-glycosylase from HeLa cells: general properties, substrate specificity and effect of uracil analogs, Nucleic Acids Res. 9 (1981) 2599–2613.
[36] J.T. Frohn, H.F. Knapp, A. Stemmer, True optical resolution beyond the Rayleigh limit achieved by standing wave illumination, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 7232–7236.
[37] T. Haug, F. Skorpen, P.A. Aas, V. Malm, C. Skjelbred, H.E. Krokan, Regulation of expression of nuclear and mitochondrial forms of human uracil-DNA glycosylase, Nucleic Acids Res. 26 (1998) 1449–1457.
[38] L.C. Olsen, R. Aasland, C.U. Wittwer, H.E. Krokan, D.E. Helland, Molecular cloning of human uracil-DNA glycosylase, a highly conserved DNA repair enzyme, EMBO J. 8 (1989) 3121–3125.
[39] Z. Wang, D.W. Mosbaugh, Uracil-DNA glycosylase inhibitor gene of bacteriophage PBS2 encodes a binding protein specific for uracil-DNA glycosylase, J. Biol. Chem. 264 (1989) 1163–1171.
[40] L. Trinkle-Mulcahy, S. Boulon, Y.W. Lam, R. Urcia, F.M. Boisvert, F. Vandermoere, N.A. Morrice, S. Swift, U. Rothbauer, H. Leonhardt, A. Lamond, Identifying specific protein interaction partners using quantitative mass spectrometry and bead proteomes, J. Cell Biol. 183 (2008) 223–239.
[41] M. Akbari, T. Visnes, H.E. Krokan, M. Otterlei, Mitochondrial base excision repair of uracil and AP sites takes place by single-nucleotide insertion and long-patch DNA synthesis, DNA Repair (Amst.) 7 (2008) 605–616.
[42] B. Kavli, M. Otterlei, G. Slupphaug, H.E. Krokan, Uracil in DNA—general mutagen, but normal intermediate in acquired immunity, DNA Repair (Amst.) 6 (2007) 505–516.
[43] T. Visnes, M. Akbari, L. Hagen, G. Slupphaug, H.E. Krokan, The rate of base excision repair of uracil is controlled by the initiating glycosylase, DNA Repair (Amst.) 7 (2008) 1869–1881.
[44] A.E. Tomkinson, D.S. Levin, Mammalian DNA ligases, Bioessays 19 (1997) 893–901.
[45] J.L. Parsons, P.S. Tait, D. Finch, I.I. Dianova, S.L. Allinson, G.L. Dianov, CHIP-mediated degradation and DNA damage-dependent stabilization regulate base excision repair proteins, Mol. Cell 29 (2008) 477–487.
[46] K. Zolghadr, O. Mortusewicz, U. Rothbauer, R. Kleinhans, H. Goehler, E.E. Wanker, M.C. Cardoso, H. Leonhardt, A fluorescent two-hybrid (F2H) assay for direct visualization of protein interactions in living cells, Mol. Cell Proteomics (2008).
[47] L.H. Thompson, K.W. Brookman, L.E. Dillehay, A.V. Carrano, J.A. Mazrimas, C.L. Mooney, J.L. Minkler, A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and an extraordinary baseline frequency of sister-chromatid exchange, Mutat. Res. 95 (1982) 427–440.

PAPER 2

Is not included due to copyright

PAPER 3

DNA Repair 11 (2012) 357–366

Contents lists available at SciVerse ScienceDirect

DNA Repair

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

The region of XRCC1 which harbours the three most common nonsynonymous polymorphic variants, is essential for the scaffolding function of XRCC1

Audun Hanssen-Bauer[a,1], Karin Solvang-Garten[a,1], Karin Margaretha Gilljam[a], Kathrin Torseth[a], David M. Wilson III[b], Mansour Akbari[a], Marit Otterlei[a,*]

[a] Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, N-7006 Trondheim, Norway
[b] Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, USA

ARTICLE INFO

Article history:
Received 16 September 2011
Received in revised form 3 January 2012
Accepted 3 January 2012
Available online 26 January 2012

Keywords:
XRCC1 Arg194Trp
XRCC1 Arg280His
XRCC1 Arg399Gln
DNA repair complexes
Micro-irradiation

ABSTRACT

XRCC1 functions as a non-enzymatic, scaffold protein in single strand break repair (SSBR) and base excision repair (BER). Here, we examine different regions of XRCC1 for their contribution to the scaffolding functions of the protein. We found that the central BRCT1 domain is essential for recruitment of XRCC1 to sites of DNA damage and DNA replication. Also, we found that ectopic expression of the region from residue 166–436 partially rescued the methyl methanesulfonate (MMS) hypersensitivity of XRCC1-deficient EM9 cells, suggesting a key role for this region in mediating DNA repair. The three most common amino acid variants of XRCC1, Arg194Trp, Arg280His and Arg399Gln, are located within the region comprising the NLS and BRCT1 domains, and these variants may be associated with increased incidence of specific types of cancer. While we could not detect differences in the intra-nuclear localization or the ability to support recruitment of POLβ or PNKP to micro-irradiated sites for these variants relative to the conservative protein, we did observe lower foci intensity after micro-irradiation and a reduced stability of the foci with the Arg280His and Arg399Gln variants, respectively. Furthermore, when challenged with MMS or hydrogen peroxide, we detected small but consistent differences in the repair profiles of cells expressing these two variants in comparison to the conservative protein.

1. Introduction

The human genome is constantly exposed to DNA damaging agents that generate abasic sites (AP sites), base damage of different types, and single strand breaks (SSBs). Repair of such lesions involves base excision repair (BER) and single strand break repair (SSBR) pathways. These pathways converge into a common pathway after the initial step of base removal by a DNA glycosylase in BER [1]. X-ray repair cross-complementing protein 1 (XRCC1) has an important role in SSBR/BER by acting as a non-enzymatic, scaffold protein [2–5]. The human *XRCC1* gene was identified by complementation of Chinese hamster ovary (CHO) cells that displayed increased sensitivity to X-rays and other DNA damaging agents, particularly those that generate SSBs and base lesions [6,7]. These cells specifically displayed reduced SSBR capacity and an increased frequency of sister chromatid exchange [8]. Recently, XRCC1 deficient cells were found to have reduced initial repair of uracil in DNA as well [2]. Notably, inefficient XRCC1 associated SSBR is reported to contribute to neurodegenerative disease in humans [4,9].

XRCC1 interacts with a number of proteins important to the SSBR/BER pathways, including the DNA glycosylases OGG1, NEIL2, NTH1, MPG, and UNG2 [2,10,11], AP endonuclease-1 (APE-1) [12], DNA polymerase β (POLβ) [13], DNA ligase IIIα (Lig IIIα) [14], proliferating cell nuclear antigen (PCNA) [15], poly (ADP-ribose) polymerase 1 (PARP-1)[16], PARP-2 [17] and polynucleotide kinase/phosphatase (PNKP) [18]. XRCC1 is also an important complex partner for aprataxin and tyrosyl-DNA phosphodiestrase (TDP1), two proteins found to be deficient in hereditary spinocerebellar ataxias [4,19,20]. XRCC1 forms dimers and oligomers that may serve as a platform for higher order complexes, and is found in complexes of significantly different sizes and composition [2,3,15,21]. Recently, we proposed a model whereby XRCC1 orchestrates SSBR/BER during S phase based on characterization of complexes isolated from S phase cells [2].

XRCC1 possesses two BRCA1 carboxyl-terminal (BRCT) phospho-protein interaction domains, BRCT1 and BRCT2. Based on in vitro data, the interaction between two XRCC1 molecules is suggested to be mediated by the central BRCT1 domain [21]. Furthermore, a functional BRCT1 domain in XRCC1 is shown to be important for SSBR during both G1 and S/G2 phases of

* Corresponding author at: Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, NO-7489 Trondheim, Norway. Tel.: +47 72573075; fax: +47 72576400.
E-mail address: marit.otterlei@ntnu.no (M. Otterlei).
[1] These authors contributed equally to this work.

1568-7864/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2012.01.001

the cell cycle and for cell survival following treatment with the alkylating agent methyl methanesulfonate (MMS) [22,23]. It is not clear whether the C-terminal BRCT2 domain contributes to complex formation between XRCC1 monomers; however, the stable interaction between XRCC1 and Lig IIIα, which is required for the stability of the ligase, is facilitated through this domain [14]. The BRCT2 domain is also involved in the XRCC1/Lig IIIα heterodimer formation [24]. Interestingly, recent data shows that Lig IIIα is dispensable for nuclear DNA repair, suggesting that XRCC1 dependent DNA repair may utilize other DNA ligases such as Lig I [25,26].

Of the reported promoter or coding single nucleotide polymorphisms (SNPs) in human *XRCC1* within the Ensembl[2] database, four have been extensively studied in relation with different types of cancer. These are: −77T>C (promoter, rs3213245), Arg194Trp (R194W, rs1799782), Arg280His (R280H, rs25489) and Arg399Gln (R399Q, rs25487). The three polymorphisms found in the coding region, R194W, R280H and R399Q, have been analysed in meta-studies for different cancer types, but so far the meta-studies have failed to provide an unambiguous relationship between the polymorphism and disease prevalence [27–30]. This could partly be a result of population dependent differences in the frequency and distribution of the SNPs, e.g. the R280H variant is more frequent in Asians (7–15%) than in Caucasians (4–9%). R280H has been reported to be associated with an increased rate of breast cancer in Asians (Odds Ratio (OR) = 2.27), but not in Caucasians [31]. Another meta-study suggests that a homozygous state in R194W increases lung cancer risk, especially in Asians, while R194W heterozygousity decreases the cancer risk of lung cancer, especially in Caucasians [32]. When assessing the cumulative evidence by means of the Venice criteria, only the −77T>C promoter polymorphism correlated with an increased risk of lung cancer at level B (moderate amount of evidence) [33]. Thus, despite the multiple reports of correlations for the different SNPs and certain cancer types, a broad consensus has proven to be elusive.

In this study we have explored different regions of XRCC1 as well as different XRCC1 SNP variants in facilitating: (1) the recruitment of XRCC1 to sites of micro-irradiation, (2) direct XRCC1–XRCC1 interaction, (3) the assembly of BER proteins at regions of micro-irradiation, (4) the localization of XRCC1 to replication foci and (5) the repair of hydrogen peroxide (H_2O_2) and MMS induced DNA damage. We found that the BRCT1 domain is key to correct intra-nuclear localization of XRCC1 to both repair and replication foci, and sufficient for a direct XRCC1–XRCC1 interaction. Furthermore, we show that the region covering the nuclear localization signal (NLS) and the BRCT1 domain (i.e. residues 166–436), is able to partially complement the repair defect of XRCC1-deficient cells after MMS treatment, but not after H_2O_2 treatment. For the three XRCC1 nonsynonymous SNP variants, we could not detect any major differences in intra-nuclear localization, but did observe small differences in the repair profiles and recruitment to and dissociation from micro-irradiated regions.

2. Materials and methods

2.1. Fluorescently tagged protein constructs

pCFP-PCNA, pXRCC1-YFP, pYFP-XRCC1, pYFP-POLβ and pYFP-PNKP have been previously described [2,15,34]. To generate N-terminal YFP-tagged deletion fragments of XRCC1, segments of the XRCC1 coding region were PCR amplified and subcloned into the pYFP-C1 vector (Clontech). In brief, XNTD was cloned into the BglII site, MD into the BglII/BamHI site and BLB into the BglII/EcoRI site (for details see [15]). CFP versions of YFP-tagged fusion proteins were prepared by switching the Age1/Not1fragment of the CFP and YFP-N1 vectors. SNPs of XRCC1; R194W, R280H and R399Q were introduced into pXRCC1-YFP by site-directed mutagenesis according to the protocol provided by the manufacturer (Stratagene) and verified by sequencing.

2.2. Cell lines

CHO EM9 (Xrcc1-deficient) cells stably expressing tagged human XRCC1 or the indicated deletion mutants were prepared by transfection (Fugene 6). Cells used in confocal imagery were cultured 16–48 h in normal media prior to use (Alpha modified MEM (Sigma–Aldrich) supplemented with 10% FCS, 250 μg/ml amphotericin B (Sigma–Aldrich), 100 μg/ml gentamycin (Invitrogen) and 1 mM glutamine (BioWhittaker)). Cells used in Comet analysis were selected for stable expression by prolonged culture in normal media supplemented with Genticine (G 418, Invitrogen), followed by cell sorting and cloning by dilution. All cells were cultured at 37 °C in a 5% carbon dioxide-humidified atmosphere. The stable cells lines were used in Comet analysis. Confocal analysis (co-localization, micro-irradition and FRET analysis) were done 16–48 h after transient transfection of CHO EM9 cells.

2.3. Preparation of cell extracts and immunoprecitipitaiton

Whole cell extracts were prepared by carefully resuspending the harvested cell pellet in 3× PCV (packed cell volume) in buffer I (20 mM HEPES-KOH pH 7.8, 100 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 20% glycerol, 0.5% NP-40, 1 mM DTT, 1× Complete protease inhibitor (Roche), and phosphatase inhibitor cocktail (PIC I and II; Sigma–Aldrich)). 2 μl Omnicleave Endonuclease (200 U/μl; Epicenter Technologies) was added before sonication. The extracts were treated with DNase/RNase (cocktail of 2 μl Omnicleave Endonuclease, 1 μl DNase (10 U/μl; Roche), 1 μl benzonase (250 U/μl; EMD), 1 μl micrococcal nuclease (100–300 U/mg; Sigma–Aldrich), and 10 μl RNase (2 mg/ml; Sigma–Aldrich) per 30 mg cell extract incubated at 37 °C for 1 h), and dialysed in buffer II (20 mM HEPES-KOH pH 7.8, 100 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 10% glycerol, 1 mM DTT, and 0.01× Complete protease inhibitor) at 4 °C for a minimum of 4 h, followed by clearance by centrifugation. XRCC1-YFP and deletion constructs were immunoprecipitated (IPed) using paramagnetic Protein-A beads (Dynal) covalently coupled to an in-house polyclonal rabbit anti-GFP antibody. The beads were incubated with cell extract for a minimum of 4 h at 4 °C in buffer II with additional Complete protease inhibitor (1×). The beads were washed in 10 mM Tris–HCl pH 7.5, 50 mM KCl and used in Western blotting analysis (WB). The expression levels of conservative (a.k.a. wild-type) and XRCC1 SNP variants were analysed directly by WB without IP.

2.4. Western analysis

The membranes were blocked in 5% low fat dry milk in PBST (PBS with 0.1% Tween 20), incubated with polyclonal anti-XRCC1 (after IP, deletion mutants) (Santa Cruz sc-11429) or mouse monoclonal anti-XRCC1 (Abcam ab-1838) (cell extracts of full length XRCC1 and SNP variants) in 1% dry milk at 4 °C overnight, and incubated 1 h in 1% dry milk with complementary HRP conjugated secondary antibodies and visualised using a Kodak Image Station 2000R.

2.5. Confocal imaging

Fluorescent images were acquired using a Zeiss LSM 510 Meta laser scanning microscope equipped with a Plan-Apochromate

[2] http://www.ensembl.org/Homo_sapiens/Gene/Variation_Gene/Table?g=ENSG00000073050.

63x/1.4 oil immersion objective. The images of live cells were acquired in growth medium, with the stage heated to 37 °C. CFP was excited at λ = 458 nm and detected at λ = 470–500 nm and YFP was excited at λ= 514 nm and detected at λ = 530–600 nm, using consecutive scans. The thickness of the slice was 1 μm. No image processing except contrast and intensity adjustments were performed

2.6. 405 nm micro-irradiation

A Zeiss 405 nm diode laser was focused through a Plan-Apochromate 63x/1.4 oil immersion objective to a diffraction-limited spot size in a Zeiss LSM 510 Meta laser scanning microscope. The 405 nm diode output was measured to 30 mW using a FieldMaster GS energy meter (Coherent Inc.) with a low power probe. We used 60 laser beam iterations (60 it) at a speed of 1.27 μs/pixel over a 50 × 2 pixel area in the cell nucleus outside nucleoli (low dose, recruits POLβ and PNKP, but not PCNA [3]), or 600 it (high dose, which recruits PCNA and FEN-1 as well). The high dose may give low levels of double-strand breaks (DSBs) as determined by staining for γH2AX [3]. Time lapse image acquisition started 1 scan prior to the micro-irradiation. Signal intensities were measured using the LSM 510 Meta operating software version 4.2. The relative signal strength of the foci were obtained by dividing average foci signal strength with average signal strength measured in a non-irradiated, equally sized region of the nucleus. Only cells with similar signal intensities were analysed. Size bars on the image equals 5 μm.

2.7. Fluorescent resonance energy transfer (FRET) measurements

FRET was measured using the sensitized emission method as previously described [35]. Briefly, N_{FRET} was calculated from mean intensities (I) within a region of interest (ROI) containing more than 75 pixels where all pixels had intensities below 250. Channel 1 (CFP) and 3 (YFP) were measured as described for imaging, and channel 2 (FRET) was excited with λ = 458 nm and detected at λ = 530–600 nm.

2.8. Single cell gel electrophoresis (Comet) assay

Exponentially growing CHO EM9 cells stably expressing XRCC1-YFP were treated with H_2O_2 (Sigma–Aldrich) or MMS (Sigma–Aldrich) in PBS for the indicated times and at the indicated concentrations, washed twice with PBS, and harvested in 4 °C cell medium. After centrifugation at 400 × *g* for 5 min, the cells were suspended in 37 °C 1% low-melt agar, mounted on a microscope slide, and immediately cooled to 4 °C. The embedded cells were lysed overnight at 4 °C in lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris–HCl, 10% DMSO, 1% Triton X-100, 17 mM NaLauroyl Sarcosine, pH 10). Alkaline (pH 13.3) single-cell gel electrophoresis was performed as described [36] except the samples were not treated with uracil DNA-glycosylase. Hundred Comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology).

3. Results

3.1. The BRCT1 domain is sufficient for recruitment of XRCC1 to sites of DNA damage

The contribution of the different domains of XRCC1 for the relocation of the protein itself, as well as for its binding partners, has not been extensively explored. To examine this issue, we transfected CHO cells deficient in XRCC1 (CHO EM9) with constructs expressing either full length XRCC1 or an XRCC1 deletion mutant tagged with YFP, and determined the region responsible for XRCC1 recruitment to sites of micro-irradiation-induced DNA damage. Fig. 1A shows a schematic of the deletion mutants and the binding regions for some key SSBR/BER proteins. The XRCC1 deletion constructs are partly overlapping and contain one or more domains of XRCC1. We micro-irradiated the cells with energies sufficient for recruitment of POLβ and PNKP, but not PCNA, to sites of irradiation; the dose of micro-irradiation applied was previously shown to not introduce DSBs [3].

All constructs except BLB were strictly localized in the nuclei, consistent with those residues (amino acids 239–266) predicted to make up the NLS [16] (Fig. 1B). Full length XRCC1 rapidly assembled into foci after micro-irradiation, showing a maximum intensity increase of approximately 2.0 fold around 100 s post irradiation. MD and BLB deletion mutants were also recruited to micro-irradiated regions, while XNTD did not move into foci even at 10–100 fold higher laser doses (Fig. 1B, marked with white arrows, and data not shown). The results above were the same for all XRCC1 deletion mutants regardless of whether the YFP tag was positioned at the N- or C-terminus (not shown). Although the total nuclear intensity of the YFP-BLB deletion mutant was comparable to YFP-MD and XRCC1-YFP, the BLB mutant foci had lower intensity; we observed a maximum of a 1.4 fold increase compared to the 2 fold increase for the full length XRCC1 and the MD deletion mutant (Fig. 1B, right panel). This result suggests that residues 166–310 are important not only for nuclear localization, but also for recruitment to and/or stability of complexes at the sites of DNA damage. The importance of the BRCT1 domain for the ability of XRCC1 to form repair foci was demonstrated as the XNTD mutant did not recruit to sites of high dose micro-irradiation.

Visual colocalization of proteins does not necessarily mean that they are directly interacting, while positive fluorescence resonance energy transfer (FRET) requires that the fluorescent tags are less than 100 Å apart [37]. In order to examine whether only BRCT1, or both BRCT-domains in XRCC1 is important for the direct XRCC1–XRCC1 interactions, we measured FRET between CFP and YFP tagged MD-constructs and compared to the full length XRCC1. FRET data showed that similar to full length XRCC1s [15], the MD constructs were closer than 100 Å (Fig. 1 C). This strongly suggests that the BRCT1 domain is capable of mediating the XRCC1–XRCC1 interaction.

3.2. Recruitment of DNA POLβ to micro-irradiated regions requires both the NTD and the BRCT1 domain, while recruitment of PCNA and PNKP is only partly affected by XRCC1

Localization of POLβ to irradiated regions is strictly dependent upon XRCC1, while recruitment of PNKP and PCNA is enhanced by XRCC1 [3]. We examined if the XRCC1 mediated recruitment of POLβ, PCNA and PNKP to micro-irradiated regions was dependent upon direct interaction to their respective binding regions (Fig. 1A), which previously have been mapped by in vitro analysis of purified proteins [13,15,18]. CHO EM9 cells were either transfected with tagged POLβ, PCNA, or PNKP alone or together with tagged full length XRCC1, MD, or BLB (CPF/YFP in pair). Because XNTD did not form foci after micro-irradiation (Fig. 1B), it was not included in these studies. Confocal analysis verified that even upon very high doses of laser micro-irradiation (1200 iterations), more than sufficient to introduce DNA DSBs, no POLβ foci could be detected in absence of XRCC1, while PNKP and PCNA were recruited to sites of damage (Table 1). POLβ was recruited to micro-irradiated regions when co-expressed with full length XRCC1, but not with the MD or BLB fragments (Appendix A, Table 1). Thus, the reported NTD binding region in XRCC1 is required for relocation of POLβ. Recruitment of PCNA to micro-irradiated regions increased in the presence of XRCC1, and this may be associated with a direct interaction

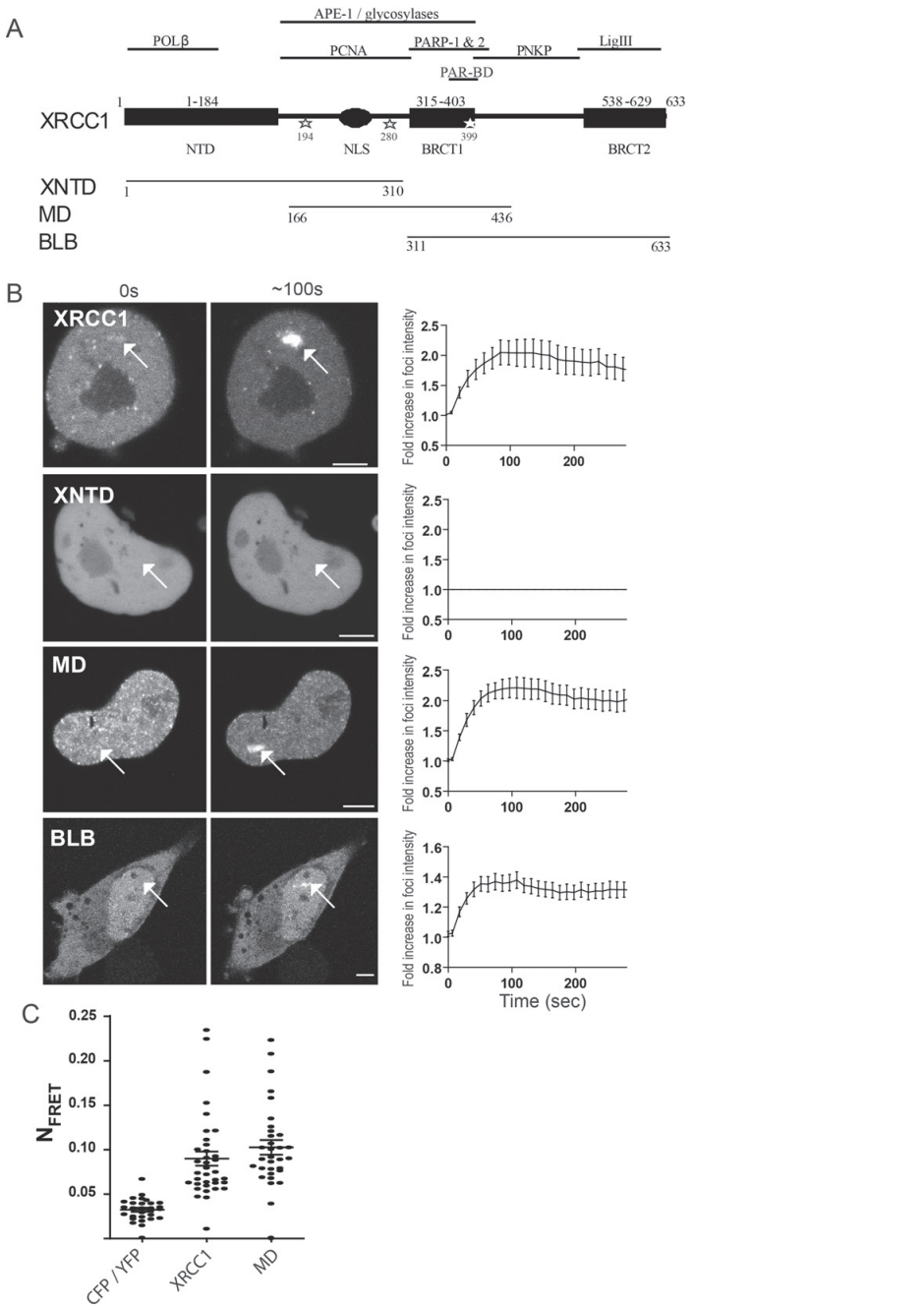


Fig. 1. Cellular localization and level of recruitment of XRCC1 deletion mutants to micro-irradiated regions. (A) Overview of the distinct domains in XRCC1, sites of interactions with other proteins, position of the three most common nonsynonymous polymorphic variants and definition of deletion mutants used in this study. XNTD: extended XRCC1 N-terminal domain, BRCT1/2: BRCA1 C-terminus (BRCT) domains. NLS: nuclear localization domain. BLB: BRCT1 Linked BRCT2 domain, MD: Mid Domain (B) Left panel: recruitment of full length XRCC1-YFP and YFP-XNTD, YFP-MD, YFP-BLB deletion mutants to sites of micro-irradiation (60 it, see Section 2); bars 5 μm. Arrows indicate irradiated regions. Right panel: graphs showing fold increase in foci intensities after micro-irradiation of the irradiated region. Mean ± SEM, XRCC1 $n = 15$, XNTD $n = 5$, MD $n = 15$, BLB $n = 20$. (C)N_{FRET} is calculated by sensitized emission as described in Section 2. Representative data from one out of there independent experiments are shown. Cells were co-transfected with CFP and YFP vectors (CFP/YFP), full length (XRCC1-CFP/XRCC1-YFP) and MD deletion mutants (CFP-MD/YFP-MD). N_{FRET} in selected regions of interest (ROI) are presented as dots with mean ± SEM, $n = 30–36$.

Table 1
Ability of XRCC1 and XRCC1 deletion mutants to support foci formation after micro-irradiation.

Cell lines	Cells transfected with indicated constructs		POLβ	PCNA	PNK
EM9 (XRCC1^{-}/$^{-}$)			–[a]	+[a]	+[a]
AA8			+++[a]	++[a]	+++[a]
EM9 (XRCC1^{-}/$^{-}$)	f.l. XRCC1	+++	+++[a]	++[a]	+++[a]
EM9 (XRCC1^{-}/$^{-}$)	XNTD	-	N.D.	N.D.	N.D.
EM9 (XRCC1^{-}/$^{-}$)	MD	+++	–	++	+
EM9 (XRCC1^{-}/$^{-}$)	BLB	+++	–	+	++

N.D., not determined; f.l., full length; –, no foci; +, foci after 1200 iterations;
++, foci after 600 iterations; +++, foci after 60 iterations.
[a] Published previously in Hanssen-Bauer et al. [3].

between XRCC1 and PCNA, as MD, but not BLB, enhanced the recruitment of PCNA to micro-irradiated regions (Table 1). Furthermore, we found that BLB, but not MD, increased localization of PNKP to sites of DNA damage generated by micro-irradiation, consistent with the direct interaction between PNKP and XRCC1 facilitating the recruitment (Table 1). CFP-tagged POLβ, PCNA, and PNKP were all recruited similarly to micro-irradiated regions in the parental XRCC1 proficient cell line (CHO AA8) used as a control (Table 1). Images corresponding to Table 1 are given in Supplemental Fig. S1.

3.3. The BRCT1 domain of XRCC1 is required for recruitment of XRCC1 to replication foci

Although XRCC1 lacks the two reported PCNA interaction motifs [35,38], XRCC1 interacts directly with PCNA and localizes to replication foci independent of an exogenous source of DNA damage [15]. XRCC1 likely operates here as part of a distinct replication associated complex proficient in BER [2]. The results presented in Table 1 show that the identified PCNA binding region in XRCC1 [15] is important for recruitment of PCNA to micro-irradiated regions. We examined whether the DNA damage independent localization of XRCC1 to replication foci was directed by the PCNA binding region in XRCC1. We found that both BLB and MD, similar to full length XRCC1, colocalized with PCNA in replication foci (Fig. 2), even though only MD includes the reported PCNA binding region (Fig. 1A). XNTD, on the other hand, which includes most of the PCNA binding region and pulls down PCNA in vitro [15], did not colocalize with replication foci. Thus, colocalization of XRCC1 and PCNA in replication foci is dependent on the region between 310 and 436 of XRCC1, which includes the BRCT1 domain. In addition, co-localization of the BLB deletion mutant and PCNA implies that XRCC1 can be recruited to replication foci via binding to proteins other than PCNA. Candidate proteins are PARP-1 [39], MPG [40] and UNG2 [2,41], which have been found in replication foci and have their XRCC1 binding domains in, or partially within, the BLB deletion mutant.

3.4. The NLS-BRCT1 region in XRCC1 is important for rapid repair of MMS induced DNA damage

XRCC1-deficient cells show an increased sensitivity to DNA damage introduced by MMS (10 fold) and H_2O_2 (2 fold) [7]. Because both MD and BLB were recruited to micro-irradiated regions and colocalized with replication foci, they could potentially scaffold repair proteins and support SSBR/BER. We examined this hypothesis in cloned cell lines of CHO EM9 cells stably expressing the deletion constructs using the Comet assay. The Comet assay as performed here enabled us to detect the total amount of DSBs, SSBs and alkali-labile sites (i.e. AP sites); hence, all repair intermediates of BER/SSBR after removal of damaged bases.

In three independent experiments we found that the MD deletion mutant, unlike the XNTD or BLB fragment, was able to partially complement XRCC1 for repair of MMS induced DNA damage (Fig. 3A show merged results). Judged by the average fluorescence intensities and Western blot analysis (Fig. 3C), this outcome was clearly not related to a higher expression level of MD compared to the other deletion mutants. The MD mutant spans the region reported to be important for interactions with DNA glycosylases (amino acids 166–436), such as MPG [10], as well as with PCNA, APE-1, and PARP-1 [12,15,16]. The MD fragment, however, lacks the portions of XRCC1 responsible for the interactions with Lig IIIα and POLβ [13,14]. Since the MD deletion mutant also contains the reported binding regions for OGG1, NTH1 and NEIL2 [10,11], DNA glycosylases primarily involved in the repair of oxidative base lesions, we tested the ability of this fragment to complement XRCC1 after treatment of cells with H_2O_2. Neither MD, nor the other deletion mutants, contributed reproducibly to any change in DNA repair after H_2O_2 treatment in parallel experiments (Fig. 3B). Thus, our results indicate that a specific interaction of the MD deletion mutant is critical for the efficient XRCC1-directed repair of MMS-induced DNA damage, yet is dispensable for oxidative DNA damage repair. We examined whether the complementation

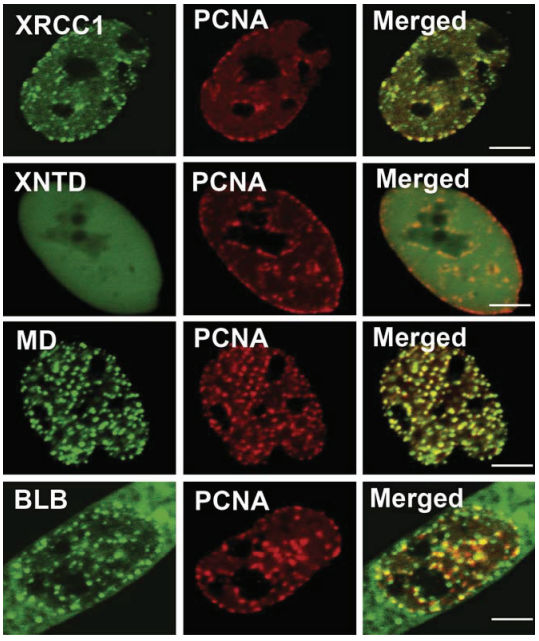


Fig. 2. Colocalization of the XRCC1 deletion mutants and PCNA in replication foci. Confocal fluorescence images of XRCC1 and its deletion mutants with YFP-tag (green) co-expressed with CFP-tagged PCNA (red) in live S phase CHO EM9 cells. The yellow dots in the merged pictures (third row) indicate colocalization of the proteins; bars 5 μm. (For interpretation of the references to color in figure caption, the reader is referred to the web version of the article.)

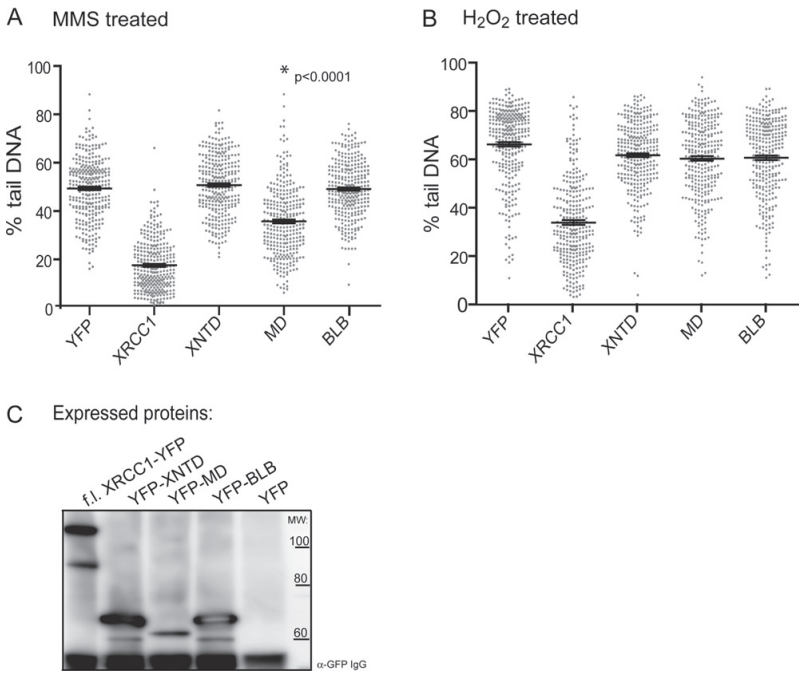


Fig. 3. Comet analysis of the XRCC1 deletion mutants for their ability to complement full length XRCC1. Analysis of CHO EM9 cells stably expressing YFP, XRCC1-YFP, YFP-XNTD, YFP-MD and YFP-BLB. Comet analysis after 10 min treatment with (A) MMS (600 μM) or (B) H_2O_2 (62.5 μM). 100 comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology). Data presented are merged from three independent experiments ($n = 100$), and are presented as scatter plot of % Tail DNA on the *y*-axis. Mean ± SEM, $n = 300$. $^*p < 0.0001$. (C) Expression levels of the fusion proteins in the different cell lines detected after immunoprecipitation (IP). Two bands are seen due to partial degradation of the fusion proteins. The band at 50 kDa represent IgG from the IP.

observed may stem from an interaction between XRCC1 and MPG, but found that knock down of MPG by siRNA did not change the pattern of repair for any of the deletion constructs (data not shown).

3.5. The variants Arg194Trp, Arg280His and Arg399Gln of XRCC1 display subtle differences in repair profiles

The MD deletion mutant exhibits similar nuclear localization to full length XRCC1, responds similarly to micro-irradiation as full length XRCC1, and partly complements the MMS hypersensitivity of the EM9 cell line. The three most frequent non-synonymous SNPs found in XRCC1, Arg194Trp (R194W), Arg280His (R280H) and Arg399Gln (R399Q), are all located within the MD fragment (marked by stars in Fig. 1A). Since this region seems to be essential for both the proper localization and the scaffolding function of XRCC1, we examined intra-nuclear localization of YFP-tagged versions of these XRCC1 polymorphic variants. All three variants showed a localization pattern similar to the conservative (a.k.a. wild-type) form of XRCC1 (Fig. 4), consistent with previous results [42]. Moreover, all three variants colocalized with PCNA in replication foci (panel A), and supported relocation of POLβ (panel B) and PNKP (panel C) to regions of micro-irradiation similar to conservative XRCC1. We note that only the R399Q XRCC1 variant tagged with YFP is shown in comparison with conservative XRCC1-YFP in Fig. 4; the other two polymorphic variants behaved similarly and are shown in Supplemental Fig. S2.

We next determined quantitatively the kinetics of appearance and dissociation of the polymorphic variants from sites of micro-irradiation relative to the conservative XRCC1. For the R194W variant, we did not detect a significantly different pattern of relocation compared to the conservative XRCC1 (Fig. 4D, left panel), although a tendency toward a lower foci intensity, hence less accumulation, was observed. The R399Q variant reached its highest foci intensity peak earlier than the conservative XRCC1 (after 60 s versus 120 s, respectively, shown in red), suggesting a reduced ability to stay at the site of damage. Moreover, the initial slope of the curve supported a more immediate dissociation of the R399Q variant (Fig. 4D, right panel), although the difference in average intensities was not statistically significant until 300 s (marked with *). For heterozygous R280H, a recent report [42] suggested that this variant dissociated more rapidly than its conservative form from sites of micro-irradiation in HeLa cells. Under our experimental conditions (homozygous expression and low dose micro-irradiation of CHO EM9 cells not introducing DSBs), we could not detect any difference in the rate of dissociation, i.e. the slopes are equal for the R280H variant compared to conservative XRCC1. However, R280H did display a tendency, which was statistically significant after 300 s, for impaired foci formation, i.e. lower foci intensity (Fig. 4D, mid panel). The time to reach maximum intensity was similar for the R280H variant and the conservative XRCC1. Finally, we detected small differences in the ability of two (R194W and R399Q) of the XRCC1 variants to complex with the conservative XRCC1 by FRET analysis (Supplemental Fig. S3).

Because reduced ability to be recruited to foci or an increased rate of dissociation could lead to repair deficiencies, we next examined the repair kinetics of MMS and H_2O_2 induced DNA damage in XRCC1-deficient cells complemented with the conservative or a variant XRCC1 protein using the Comet assay as described earlier. In order to detect subtle differences in repair efficacy between the conservative XRCC1 and polymorphic variants, we used high

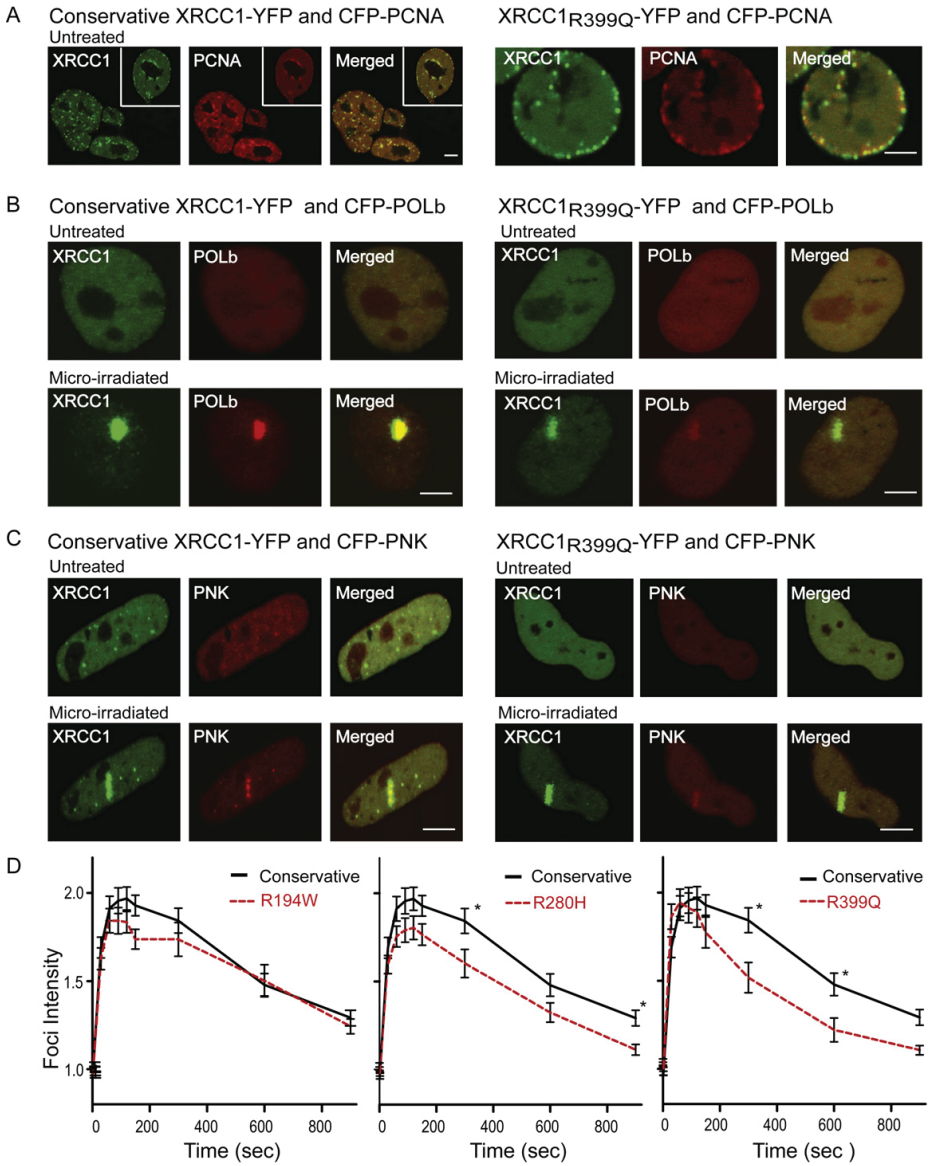


Fig. 4. Colocalization analysis of XRCC1 or XRCC1 SNP variants, with POLβ and PNKP in micro-irradiated regions, and with PCNA in replication foci. (A) CHO EM9 cells co-transfected with conservative XRCC1-YFP (left panel) and XRCC1$_{R399Q}$-YFP (right panel, R399Q is representative for all three variants) with CFP-PCNA, (B) conservative XRCC1-YFP (left panel) and XRCC1$_{R399Q}$-YFP (right panel, R399Q is representative for all three variants) with CFP-POLβ (C) conservative XRCC1-YFP (left panel) and XRCC1$_{R399Q}$-YFP (right panel, R399Q is representative for all three variants) with CFP-PNKP; bars 5 μm. (D) Graphs showing recruitment and dissociation of XRCC1-YFP and XRCC1$_{R194W}$-YFP (left panel), XRCC1$_{R280H}$-YFP (mid panel) and XRCC1$_{R399Q}$-YFP (right panel) to selected micro-irradiated regions (60 it, see Section 2). Graphs show mean ± SEM, conservative XRCC1-YFP, $n = 28$ (from three independent experiments), XRCC1$_{R194W}$-YFP, $n = 10$, XRCC1$_{R280H}$-YFP, $n = 14$ and XRCC1$_{R399Q}$-YFP (in red), $n = 14$. $^*p < 0.05$. (For interpretation of the references to color in figure caption, the reader is referred to the web version of the article.)

doses of MMS and H_2O_2 (8 and 4 fold, respectively, higher than in experiments shown in Fig. 3). After 120 min, the DNA damage levels were reduced for each of the complemented cell lines, indicating that all cells were able to recover after these doses of MMS and H_2O_2 (Fig. 5A). The polymorphic variants, however, showed significant and reproducible differences in the pattern of % tail DNA compared to the conservative XRCC1 after MMS treatment; initially lower tail. A disparate pattern was also seen for the R280H and R399Q variants after H_2O_2 treatment (Fig. 5A, upper row shows comet tail distribution in one representative experiment, two other experiments are shown in Supplemental Fig. S4). Western analysis (Fig. 5B) showed no correlation between the DNA repair profile and the expression level of the polymorphic variants relative to the conservative XRCC1 protein.

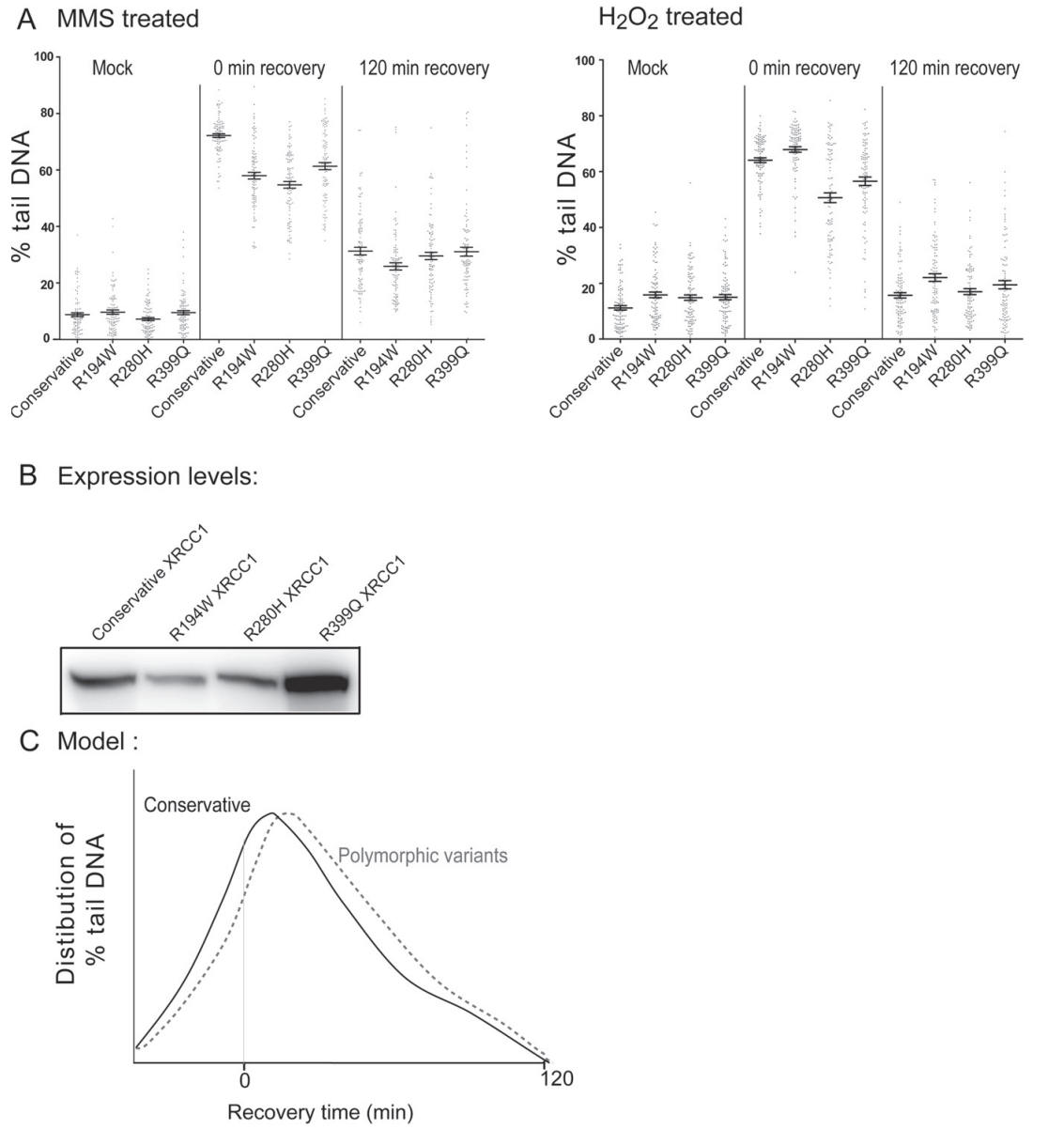


Fig. 5. Analysis of the ability of the XRCC1 SNP variants to complement conservative XRCC1. (A) Comet analysis of CHO EM9 cells stably expressing XRCC1-YFP, XRCC1$_{R194W}$-YFP, XRCC1$_{R280H}$-YFP, XRCC1$_{R399Q}$-YFP after treatment with MMS (4.8 mM) (left panel) and H_2O_2 (250 μM) (right panel) after 0 and 120 min recovery. 100 comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology). Data presented is from one representative out of three independent experiments, and is presented as scatter plot of % tail DNA on the *y*-axis. Mean ± SEM, $n = 100$ (B) expression levels of the fusion proteins in the corresponding cell lines (C) model of different repair kinetics between conservative and polymorphic variants. Dashed grey line represents polymorphic variants with delayed BER.

4. Discussion

Point mutations in the BRCT1 domain of XRCC1 (i.e. LI360/361DD and W285D) are reported to affect cellular resistance to MMS induced DNA damage. However, the intra-nuclear localization of the described BRCT1 mutants is not known. Since these mutations disrupt the correct folding of XRCC1, it is possible that the amino acid substitutions may in fact impair proper intra-cellular distribution [22,23,43]. Because expression of the BLB-mutant does not rescue the XRCC1-deficient cells, our data suggest that it is not the BRCT1 domain per se, but the larger NLS-BRCT1 region (residue 166–436) that is important for the ability of XRCC1 to support repair after MMS treatment.

We have recently published that localization of POLβ to sites of irradiation was completely dependent on XRCC1, while the recruitment of PNKP and PCNA was only enhanced in the presence of

XRCC1 [3]. Studies have shown that XRCC1 binds to nicked and gapped DNA [44] and that the NTD domain of XRCC1 exhibits a specific affinity for these lesions [45]. This domain of XRCC1 (NTD) also interacts with POLβ [13]. A previous study suggests a POLβ dependent recruitment of XRCC1 to sites of DNA damage [46]. However, the fact that the XNTD deletion mutant does not form foci suggests that the interaction with POLβ is not sufficient for re-localization of XRCC1 to sites of damage.

XRCC1 and the three polymorphic variants, R194W, R280H and R399Q, show similar intra-nuclear localization, and all variants supported POLβ and PNKP relocation to sites of micro-irradiation. Interestingly, the R399Q variant is located within a conserved PAR-binding motif (amino acids 379–400 [47]) (see Fig. 1A). We found that this variant had a reduced ability to remain at sites of micro-irradiation, and this could possibly be due to reduced PAR-binding. Reduced XRCC1 recruitment and/or ability to form stable complexes will likely affect recruitment of XRCC1 interacting proteins and the overall efficiency of repair.

The Comet assay as performed here detects AP sites, SSB and DSBs and several forms of repair intermediates, but not damaged bases. The differences in the repair profiles of the XRCC1 variants after MMS and H_2O_2 treatment could be explained by reduced recruitment of these XRCC1s and interacting proteins to sites of DNA damage, reduced ability to make complexes or interact with DNA glycosylases, and/or reduced efficiency of excision of damaged bases or resolution of strand break intermediates. Reduced base excision will result in less % tail DNA immediately or shortly after DNA damage introduction, which is what we see (Fig. 5A). Thus, what may be interpreted as more efficient initial repair by merely looking at the comet tails, could actually be a delayed BER response as illustrated in the model in Fig. 5C (broken line). Such differences may only be detectable in a small time window and may vary with different types of DNA damage. Interestingly, NSCLC (non-small cell lung cancer) patients, who are heterozygous for the XRCC1 variants R280H and R399Q, were found to have significantly lower 8-oxoG incision activity in extracts from lung tissue compared to extracts from NSCLC patients homozygous for the conservative form of XRCC1 [48]. Because XRCC1 forms multimeric complexes, reduced ability of the variants for complex stability (Fig. 4D and Supplemental Fig. S3) could possibly affect repair also in heterozygote variants.

Recent reports concerning a possible association of XRCC1 variants with cancer incidence have detected high OR values when including several variants within the XRCC1 protein or when combined with SNPs in other DNA repair proteins. For instance, R399Q was reported to be associated with increased breast cancer risk alone (OR = 4.67) and together with the −77T>C variant (OR = 7.04) [49]. R399Q was furthermore found to be associated with an increased colorectal cancer risk alone (OR = 1.65), while a significantly stronger association was found in combination with a XPD SNP (Lys751Asn) (subunit of transcription factor IIH, involved in nucleotide excision repair, NER) (OR = 3.52) [50]. Thus, even if the differences in the repair profiles of the SNPs detected under our experimental conditions are subtle, they might be of biological significance, and perhaps more so when combined with polymorphic variants of other DNA repair proteins in the same pathway.

In summary, in this paper we have shown that the region in XRCC1 harbouring the three most common SNPs is essential for localization of XRCC1 and its interacting partners to sites of DNA damage and DNA replication. Although we did not observe profound differences in the functional activities of the XRCC1 polymorphic variants, there was a statistically significant reduction in the ability of R280H to be recruited to or R399Q to remain at foci after micro-irradiation. A significant change in repair profile after DNA damage induced by H_2O_2 and MMS was also observed with the R280H and R399Q variant-complemented EM9 cell lines. The observed differences herein may ultimately affect the overall regulation of BER/SSBR and account for the reported associations of XRCC1 polymorphisms with increased risk of disease.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We would like to thank Nina-Beate Liabakk for technical assistance. This work is supported by The Cancer Fund at St. Olavs Hospital, Trondheim, Norway, The Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU), Norway, The Norwegian Research Council program FUGE, The Norwegian Cancer Society, and the Intramural Research Program of the NIH, National Institute on Aging, USA.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.dnarep.2012.01.001.

References

[1] D.O. Zharkov, Base excision DNA repair, Cell Mol. Life Sci. 65 (2008) 1544–1565.
[2] M. Akbari, K. Solvang-Garten, A. Hanssen-Bauer, N.V. Lieske, H.S. Pettersen, G.K. Pettersen, D.M. Wilson 3rd., H.E. Krokan, M. Otterlei, Direct interaction between XRCC1 and UNG2 facilitates rapid repair of uracil in DNA by XRCC1 complexes, DNA Repair (Amst.) 9 (2010) 785–795.
[3] A. Hanssen-Bauer, K. Solvang-Garten, O. Sundheim, J. Pena-Diaz, S. Andersen, G. Slupphaug, H.E. Krokan, D.M. Wilson 3rd., M. Akbari, M. Otterlei, XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage, Environ. Mol. Mutagen. 52 (2011) 623–635.
[4] S.F. El-Khamisy, G.M. Saifi, M. Weinfeld, F. Johansson, T. Helleday, J.R. Lupski, K.W. Caldecott, Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1, Nature 434 (2005) 108–113.
[5] H. Luo, D.W. Chan, T. Yang, M. Rodriguez, B.P. Chen, M. Leng, J.J. Mu, D. Chen, Z. Songyang, Y. Wang, J. Qin, A new XRCC1-containing complex and its role in cellular survival of methyl methanesulfonate treatment, Mol. Cell Biol. 24 (2004) 8356–8365.
[6] L.H. Thompson, K.W. Brookman, N.J. Jones, S.A. Allen, A.V. Carrano, Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange, Mol. Cell Biol. 10 (1990) 6160–6171.
[7] L.H. Thompson, K.W. Brookman, L.E. Dillehay, A.V. Carrano, J.A. Mazrimas, C.L. Mooney, J.L. Minkler, A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and an extraordinary baseline frequency of sister-chromatid exchange, Mutat. Res. 95 (1982) 427–440.
[8] K.W. Caldecott, Mammalian DNA single-strand break repair: an X-ra(y)ted affair, Bioessays 23 (2001) 447–455.
[9] M. Hirano, A. Yamamoto, T. Mori, L. Lan, T.A. Iwamoto, M. Aoki, K. Shimada, Y. Furiya, S. Kariya, H. Asai, A. Yasui, T. Nishiwaki, K. Imoto, N. Kobayashi, T. Kiriyama, T. Nagata, N. Konishi, Y. Itoyama, S. Ueno, DNA single-strand break repair is impaired in aprataxin-related ataxia, Ann. Neurol. 61 (2007) 162–174.
[10] A. Campalans, S. Marsin, Y. Nakabeppu, R. O'Connor T, S. Boiteux, J.P. Radicella, XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair, DNA Repair (Amst.) 4 (2005) 826–835.
[11] S. Marsin, A.E. Vidal, M. Sossou, J. Menissier-de Murcia, F. Le Page, S. Boiteux, G. de Murcia, J.P. Radicella, Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1, J. Biol. Chem. 278 (2003) 44068–44074.
[12] A.E. Vidal, S. Boiteux, I.D. Hickson, J.P. Radicella, XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein–protein interactions, EMBO J. 20 (2001) 6530–6539.
[13] K.W. Caldecott, S. Aoufouchi, P. Johnson, S. Shall, 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 Res. 24 (1996) 4387–4394.
[14] K.W. Caldecott, C.K. McKeown, J.D. Tucker, S. Ljungquist, L.H. Thompson, An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III, Mol. Cell Biol. 14 (1994) 68–76.
[15] J. Fan, M. Otterlei, H.K. Wong, A.E. Tomkinson, D.M. Wilson 3rd., XRCC1 co-localizes and physically interacts with PCNA, Nucleic Acids Res. 32 (2004) 2193–2201.
[16] M. Masson, C. Niedergang, V. Schreiber, S. Muller, J. Menissier-de Murcia, G. de Murcia, XRCC1 is specifically associated with poly(ADP-ribose) polymerase

and negatively regulates its activity following DNA damage, Mol. Cell Biol. 18 (1998) 3563–3571.
[17] V. Schreiber, J.C. Ame, P. Dolle, I. Schultz, B. Rinaldi, V. Fraulob, J. Menissier-de Murcia, G. de Murcia, Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1, J. Biol. Chem. 277 (2002) 23028–23036.
[18] C.J. Whitehouse, R.M. Taylor, A. Thistlethwaite, H. Zhang, F. Karimi-Busheri, D.D. Lasko, M. Weinfeld, K.W. Caldecott, XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair, Cell 104 (2001) 107–117.
[19] I. Plo, Z.Y. Liao, J.M. Barcelo, G. Kohlhagen, K.W. Caldecott, M. Weinfeld, Y. Pommier, Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions, DNA Repair (Amst.) 2 (2003) 1087–1100.
[20] I. Ahel, U. Rass, S.F. El-Khamisy, S. Katyal, P.M. Clements, P.J. McKinnon, K.W. Caldecott, S.C. West, The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates, Nature 443 (2006) 713–716.
[21] P.T. Beernink, M. Hwang, M. Ramirez, M.B. Murphy, S.A. Doyle, M.P. Thelen, Specificity of protein interactions mediated by BRCT domains of the XRCC1 DNA repair protein, J. Biol. Chem. 280 (2005) 30206–30213.
[22] R.M. Taylor, A. Thistlethwaite, K.W. Caldecott, Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair, Mol. Cell Biol. 22 (2002) 2556–2563.
[23] Y. Kubota, S. Horiuchi, Independent roles of XRCC1's two BRCT motifs in recovery from methylation damage, DNA Repair (Amst.) 2 (2003) 407–415.
[24] M.J. Cuneo, S.A. Gabel, J.M. Krahn, M.A. Ricker, R.E. London, The structural basis for partitioning of the XRCC1/DNA ligase III-{alpha} BRCT-mediated dimer complexes, Nucleic Acids Res. 39 (2011) 7816–7827.
[25] D. Simsek, A. Furda, Y. Gao, J. Artus, E. Brunet, A.K. Hadjantonakis, B. Van Houten, S. Shuman, P.J. McKinnon, M. Jasin, Crucial role for DNA ligase III in mitochondria but not in XRCC1-dependent repair, Nature 471 (2011) 245–248.
[26] Y. Gao, S. Katyal, Y. Lee, J. Zhao, J.E. Rehg, H.R. Russell, P.J. McKinnon, DNA ligase III is critical for mtDNA integrity but not XRCC1-mediated nuclear DNA repair, Nature 471 (2011) 240–244.
[27] A. Gsur, K. Bernhart, A. Baierl, E. Feik, G. Fuhrlinger, P. Hofer, G. Leeb, K. Mach, M. Micksche, No association of XRCC1 polymorphisms Arg194Trp and Arg399Gln with colorectal cancer risk, Cancer Epidemiol. 35 (2011) 38–41.
[28] H. Xue, P. Ni, B. Lin, H. Xu, G. Huang, X-ray repair cross-complementing group 1 (XRCC1) genetic polymorphisms and gastric cancer risk: a HuGE review and meta-analysis, Am. J. Epidemiol. 173 (2011) 363–375.
[29] B. Chen, Y. Zhou, P. Yang, X.T. Wu, Polymorphisms of XRCC1 and gastric cancer susceptibility: a meta-analysis, Mol. Biol. Rep. 39 (2012) 1305–1313.
[30] J. Huang, J. Zhang, Y. Zhao, B. Liao, J. Liu, L. Li, M. Liao, L. Wang, The Arg194Trp polymorphism in the XRCC1 gene and cancer risk in Chinese mainland population: a meta-analysis, Mol. Biol. Rep. 38 (2011) 4565–4573.
[31] H. Li, T.C. Ha, B.C. Tai, XRCC1 gene polymorphisms and breast cancer risk in different populations: a meta-analysis, Breast 18 (2009) 183–191.
[32] J. Jiang, X. Liang, X. Zhou, R. Huang, Z. Chu, Q. Zhan, H. Lin, DNA repair gene X-ray repair cross complementing group 1 Arg194Trp polymorphism on the risk of lung cancer: a meta-analysis on 22 studies, J. Thorac. Oncol. 5 (2010) 1741–1747.
[33] P. Vineis, M. Manuguerra, F.K. Kavvoura, S. Guarrera, A. Allione, F. Rosa, A. Di Gregorio, S. Polidoro, F. Saletta, J.P. Ioannidis, G. Matullo, A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility, J. Natl. Cancer Inst. 101 (2009) 24–36.
[34] P.A. Aas, M. Otterlei, P.O. Falnes, C.B. Vagbo, F. Skorpen, M. Akbari, O. Sundheim, M. Bjoras, G. Slupphaug, E. Seeberg, H.E. Krokan, Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA, Nature 421 (2003) 859–863.
[35] K.M. Gilljam, E. Feyzi, P.A. Aas, M.M. Sousa, R. Muller, C.B. Vagbo, T.C. Catterall, N.B. Liabakk, G. Slupphaug, F. Drablos, H.E. Krokan, M. Otterlei, Identification of a novel, widespread, and functionally important PCNA-binding motif, J. Cell Biol. 186 (2009) 645–654.
[36] H. Nilsen, I. Rosewell, P. Robins, C.F. Skjelbred, S. Andersen, G. Slupphaug, G. Daly, H.E. Krokan, T. Lindahl, D.E. Barnes, Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication, Mol. Cell 5 (2000) 1059–1065.
[37] L. Matyus, Fluorescence resonance energy transfer measurements on cell surfaces. A spectroscopic tool for determining protein interactions, J. Photochem. Photobiol. B 12 (1992) 323–337.
[38] E. Warbrick, The puzzle of PCNA's many partners, Bioessays 22 (2000) 997–1006.
[39] H.E. Bryant, E. Petermann, N. Schultz, A.S. Jemth, O. Loseva, N. Issaeva, F. Johansson, S. Fernandez, P. McGlynn, T. Helleday, PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination, EMBO J. 28 (2009) 2601–2615.
[40] L. Xia, L. Zheng, H.W. Lee, S.E. Bates, L. Federico, B. Shen, T.R. O'Connor, Human 3-methyladenine-DNA glycosylase: effect of sequence context on excision, association with PCNA, and stimulation by AP endonuclease, J. Mol. Biol. 346 (2005) 1259–1274.
[41] M. Otterlei, E. Warbrick, T.A. Nagelhus, T. Haug, G. Slupphaug, M. Akbari, P.A. Aas, K. Steinsbekk, O. Bakke, H.E. Krokan, Post-replicative base excision repair in replication foci, EMBO J. 18 (1999) 3834–3844.
[42] B.R. Berquist, D.K. Singh, J. Fan, D. Kim, E. Gillenwater, A. Kulkarni, V.A. Bohr, E.J. Ackerman, A.E. Tomkinson, D.M. Wilson 3rd., Functional capacity of XRCC1 protein variants identified in DNA repair-deficient Chinese hamster ovary cell lines and the human population, Nucleic Acids Res. 38 (2010) 5023–5035.
[43] R.M. Taylor, D.J. Moore, J. Whitehouse, P. Johnson, K.W. Caldecott, A cell cycle-specific requirement for the XRCC1 BRCT II domain during mammalian DNA strand break repair, Mol. Cell Biol. 20 (2000) 735–740.
[44] R.S. Mani, F. Karimi-Busheri, M. Fanta, K.W. Caldecott, C.E. Cass, M. Weinfeld, Biophysical characterization of human XRCC1 and its binding to damaged and undamaged DNA, Biochemistry 43 (2004) 16505–16514.
[45] A. Marintchev, A. Robertson, E.K. Dimitriadis, R. Prasad, S.H. Wilson, G.P. Mullen, Domain specific interaction in the XRCC1-DNA polymerase beta complex, Nucleic Acids Res. 28 (2000) 2049–2059.
[46] J.L. Parsons, I.I. Dianova, S.L. Allinson, G.L. Dianov, DNA polymerase beta promotes recruitment of DNA ligase III α-XRCC1 to sites of base excision repair, Biochemistry 44 (2005) 10613–10619.
[47] M. Stilmann, M. Hinz, S.C. Arslan, A. Zimmer, V. Schreiber, C. Scheidereit, A nuclear poly(ADP-ribose)-dependent signalosome confers DNA damage-induced IkappaB kinase activation, Mol. Cell 36 (2009) 365–378.
[48] M. Swoboda, T. Fordell, K. Klunder, J.M. Dahlstrom, M. Miranda, C. Buth, K.J. Schafer, J. Mauritsson, A. L'Huillier, M. Gisselbrecht, Phase measurement of resonant two-photon ionization in helium, Phys. Rev. Lett. 104 (2010) 103003.
[49] S. Sterpone, V. Mastellone, L. Padua, F. Novelli, C. Patrono, T. Cornetta, D. Giammarino, V. Donato, A. Testa, R. Cozzi, Single-nucleotide polymorphisms in BER and HRR genes, XRCC1 haplotypes and breast cancer risk in Caucasian women, J. Cancer Res. Clin. Oncol. 136 (2010) 631–636.
[50] J. Wang, Y. Zhao, J. Jiang, V. Gajalakshmi, K. Kuriki, S. Nakamura, S. Akasaka, H. Ishikawa, S. Suzuki, T. Nagaya, S. Tokudome, Polymorphisms in DNA repair genes XRCC1, XRCC3 and XPD, and colorectal cancer risk: a case-control study in an Indian population, J. Cancer Res. Clin. Oncol. 136 (2010) 1517–1525.

Supplemental data

Figure S1

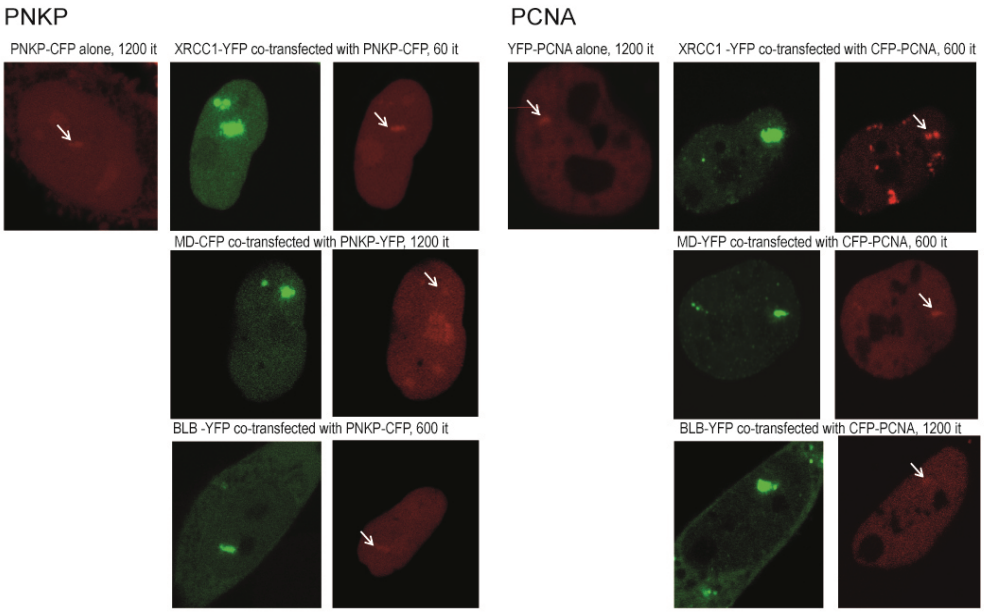


Fig. S1: Images corresponding to data shown in Table 1 (part with grey background). Lowest dose that gives detectable foci are shown.

Micro-irradiation: a Zeiss 405 nm diode laser was focused through a Plan-Apochromate 63x/1.4 oil immersion objective to a diffraction-limited spot size in a Zeiss LSM 510 Meta laser scanning microscope. The 405 nm diode output was measured to 30 mW using a FieldMaster GS energy meter (Coherent Inc.) with a low power probe. We used 60 laser beam iterations (60 it) at a speed of 1.27 µsec/pixel over a 50 × 2 pixel area in the cell nucleus outside nucleoli (CHO EM9 cells). Low dose, recruits POLβ and PNKP, but not PCNA ,600 it (high dose) recruits PCNA and FEN-1 in the presence of full length XRCC1 [1],. The highest doses (600 it and 1200 it) may give double-strand breaks (DSBs) as determined by staining for γH2AX [1].

Figure S2:

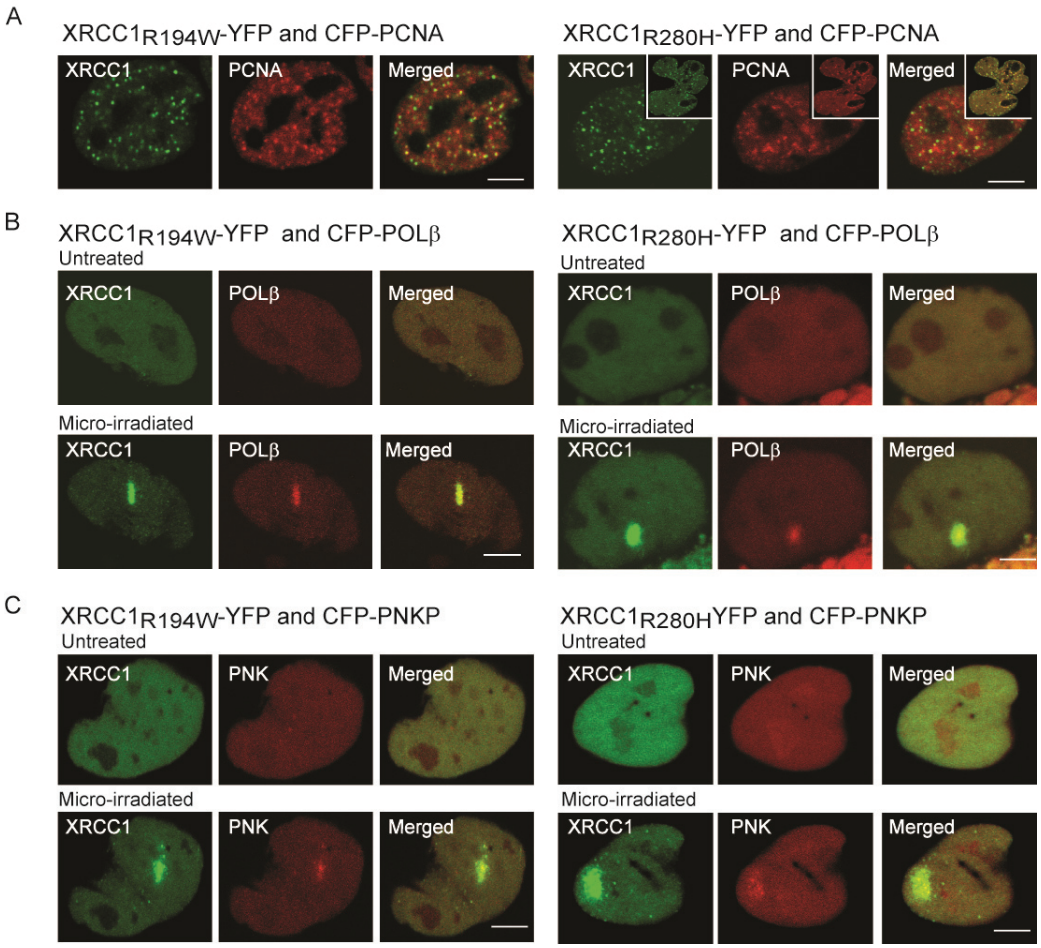


Fig. S2: Colocalization analysis of XRCC1 or XRCC1 SNP variants, with POLβ and PNKP in micro-irradiated regions, and with PCNA in replication foci. (A) CHO EM9 cells were transiently co-transfected with $XRCC1_{R194W}$-YFP (left panel) and $XRCC1_{R280H}$-YFP (right panel), with CFP-PCNA, (B) with CFP-POLβ and (C) with CFP-PNKP.

Micro-irradiation: a Zeiss 405 nm diode laser was focused through a Plan-Apochromate 63x/1.4 oil immersion objective to a diffraction-limited spot size in a Zeiss LSM 510 Meta laser scanning microscope. The 405 nm diode output was measured to 30 mW using a FieldMaster GS energy meter (Coherent Inc.) with a low power probe. We used 60 laser beam iterations (60 it) at a speed of 1.27 µsec/pixel over a 50 × 2 pixel area in the cell nucleus outside nucleoli. Bars 5 µm.

Figure S3

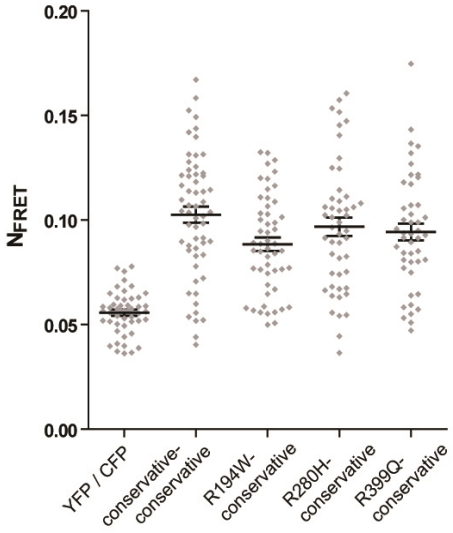


Fig. S3: N_{FRET} between conservative-conservative XRCC1, R194W-conservative XRCC1, R280H-conservative XRCC1 and R399Q-conservative XRCC1. Cells were co-transfected with CFP and YFP vectors (YFP / CFP) as a negative control. N_{FRET} in selected regions of interest (ROI) (i.e. spontaneous foci) are presented as dots with mean ± SEM, n=49-60.
Statistics:
EY/EC vs. XRCC1/XRCC1, different, $p<0.0001$
EY/EC vs. SNPs/XRCC1 (all three), different, $p<0.0001$
XRCC1/XRCC1 vs. R194W/XRCC1, different, $p=0.0008$
XRCC1/XRCC1 vs. R280H/XRCC1, not different, $p=0.1153$
XRCC1/XRCC1 vs. R399Q/XRCC1, different, $p=0.0243$

Fluorescent resonance energy transfer (FRET) measurements. Normalized FRET (N_{FRET}) was measured using the sensitized emission method as previously described [2, 3]. Briefly, N_{FRET} was calculated from mean intensities (I) within a region of interest (ROI) containing more than 75 pixels where all pixels had intensities below 250. Channel 1 (CFP) and 3 (YFP) were measured as described for imaging, and channel 2 (FRET) was excited with λ= 458 nm and detected at λ= 530-600 nm.

Figure S4

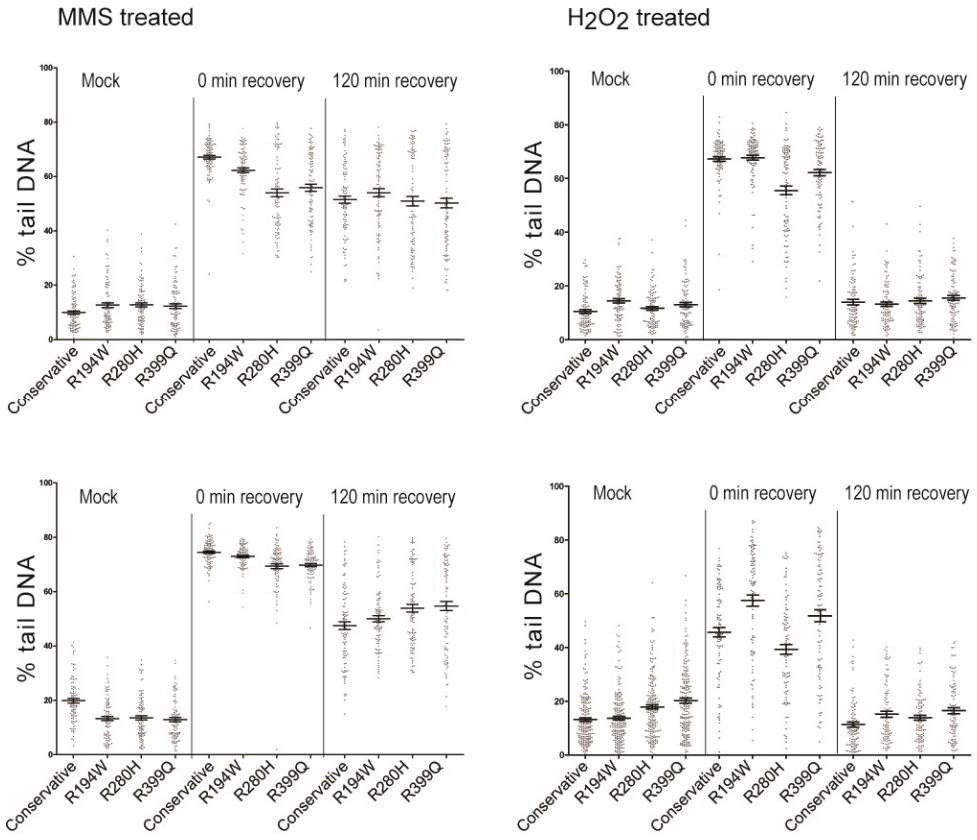


Fig. S4: Analysis of the ability of the XRCC1 SNP variants to complement conservative XRCC1. Comet analysis of CHO EM9 cells stably expressing XRCC1-YFP, XRCC1$_{R194W}$-YFP, XRCC1$_{R280H}$-YFP, XRCC1$_{R399Q}$-YFP after treatment with MMS (4.8 mM) (left panel) and H_2O_2 (250 μM) (right panel) after 0 and 120 min recovery. 100 comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology). Data presented is from two representatives out of three independent experiments, and is presented as scatter plot of % tail DNA on the y-axis. Mean ± SEM, n=100.

Comet analysis: Alkaline (pH 13.3) single-cell gel electrophoresis was performed as described [4] except the samples were not treated with uracil DNA-glycosylase.

References:

[1] A. Hanssen-Bauer, K. Solvang-Garten, O. Sundheim, J. Pena-Diaz, S. Andersen, G. Slupphaug, H.E. Krokan, D.M. Wilson, 3rd, M. Akbari, M. Otterlei, XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage, Environ Mol Mutagen, (2011).

[2] K. Baynton, M. Otterlei, M. Bjoras, C. von Kobbe, V.A. Bohr, E. Seeberg, WRN interacts physically and functionally with the recombination mediator protein RAD52, J. Biol. Chem., 278 (2003) 36476-36486.
[3] K.M. Gilljam, R. Müller, N.B. Liabakk, M. Otterlei, A direct interaction between XPA and PCNA is required for an optimal response to UV-induced DNA damage, *Submitted*, (2011).
[4] H. Nilsen, I. Rosewell, P. Robins, C.F. Skjelbred, S. Andersen, G. Slupphaug, G. Daly, H.E. Krokan, T. Lindahl, D.E. Barnes, Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication, Mol. Cell, 5 (2000) 1059-1065.

PAPER 4

Is not included due to copyright

Dissertations at the Faculty of Medicine, NTNU

1977

1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

1978

3. Karel Bjørn Cyvin: CONGENITAL DISLOCATION OF THE HIP JOINT.
4. Alf O. Brubakk: METHODS FOR STUDYING FLOW DYNAMICS IN THE LEFT VENTRICLE AND THE AORTA IN MAN.

1979

5. Geirmund Unsgaard: CYTOSTATIC AND IMMUNOREGULATORY ABILITIES OF HUMAN BLOOD MONOCYTES CULTURED IN VITRO

1980

6. Størker Jørstad: URAEMIC TOXINS
7. Arne Olav Jenssen: SOME RHEOLOGICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF MUCOID SPUTUM FROM PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHITIS

1981

8. Jens Hammerstrøm: CYTOSTATIC AND CYTOLYTIC ACTIVITY OF HUMAN MONOCYTES AND EFFUSION MACROPHAGES AGAINST TUMOR CELLS *IN VITRO*

1983

9. Tore Syversen: EFFECTS OF METHYLMERCURY ON RAT BRAIN PROTEIN.
10. Torbjørn Iversen: SQUAMOUS CELL CARCINOMA OF THE VULVA.

1984

11. Tor-Erik Widerøe: ASPECTS OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS.
12. Anton Hole: ALTERATIONS OF MONOCYTE AND LYMPHOCYTE FUNCTIONS IN REALTION TO SURGERY UNDER EPIDURAL OR GENERAL ANAESTHESIA.
13. Terje Terjesen: FRACTURE HEALING AND STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
14. Carsten Saunte: CLUSTER HEADACHE SYNDROME.
15. Inggard Lereim: TRAFFIC ACCIDENTS AND THEIR CONSEQUENCES.
16. Bjørn Magne Eggen: STUDIES IN CYTOTOXICITY IN HUMAN ADHERENT MONONUCLEAR BLOOD CELLS.
17. Trond Haug: FACTORS REGULATING BEHAVIORAL EFFECTS OG DRUGS.

1985

18. Sven Erik Gisvold: RESUSCITATION AFTER COMPLETE GLOBAL BRAIN ISCHEMIA.
19. Terje Espevik: THE CYTOSKELETON OF HUMAN MONOCYTES.
20. Lars Bevanger: STUDIES OF THE Ibc (c) PROTEIN ANTIGENS OF GROUP B STREPTOCOCCI.
21. Ole-Jan Iversen: RETROVIRUS-LIKE PARTICLES IN THE PATHOGENESIS OF PSORIASIS.
22. Lasse Eriksen: EVALUATION AND TREATMENT OF ALCOHOL DEPENDENT BEHAVIOUR.
23. Per I. Lundmo: ANDROGEN METABOLISM IN THE PROSTATE.

1986

24. Dagfinn Berntzen: ANALYSIS AND MANAGEMENT OF EXPERIMENTAL AND CLINICAL PAIN.
25. Odd Arnold Kildahl-Andersen: PRODUCTION AND CHARACTERIZATION OF MONOCYTE-DERIVED CYTOTOXIN AND ITS ROLE IN MONOCYTE-MEDIATED CYTOTOXICITY.
26. Ola Dale: VOLATILE ANAESTHETICS.

1987

27. Per Martin Kleveland: STUDIES ON GASTRIN.
28. Audun N. Øksendal: THE CALCIUM PARADOX AND THE HEART.
29. Vilhjalmur R. Finsen: HIP FRACTURES

1988

30. Rigmor Austgulen: TUMOR NECROSIS FACTOR: A MONOCYTE-DERIVED REGULATOR OF CELLULAR GROWTH.
31. Tom-Harald Edna: HEAD INJURIES ADMITTED TO HOSPITAL.
32. Joseph D. Borsi: NEW ASPECTS OF THE CLINICAL PHARMACOKINETICS OF METHOTREXATE.
33. Olav F. M. Sellevold: GLUCOCORTICOIDS IN MYOCARDIAL PROTECTION.
34. Terje Skjærpe: NONINVASIVE QUANTITATION OF GLOBAL PARAMETERS ON LEFT VENTRICULAR FUNCTION: THE SYSTOLIC PULMONARY ARTERY PRESSURE AND CARDIAC OUTPUT.
35. Eyvind Rødahl: STUDIES OF IMMUNE COMPLEXES AND RETROVIRUS-LIKE ANTIGENS IN PATIENTS WITH ANKYLOSING SPONDYLITIS.
36. Ketil Thorstensen: STUDIES ON THE MECHANISMS OF CELLULAR UPTAKE OF IRON FROM TRANSFERRIN.
37. Anna Midelfart: STUDIES OF THE MECHANISMS OF ION AND FLUID TRANSPORT IN THE BOVINE CORNEA.
38. Eirik Helseth: GROWTH AND PLASMINOGEN ACTIVATOR ACTIVITY OF HUMAN GLIOMAS AND BRAIN METASTASES - WITH SPECIAL REFERENCE TO TRANSFORMING GROWTH FACTOR BETA AND THE EPIDERMAL GROWTH FACTOR RECEPTOR.
39. Petter C. Borchgrevink: MAGNESIUM AND THE ISCHEMIC HEART.
40. Kjell-Arne Rein: THE EFFECT OF EXTRACORPOREAL CIRCULATION ON SUBCUTANEOUS TRANSCAPILLARY FLUID BALANCE.
41. Arne Kristian Sandvik: RAT GASTRIC HISTAMINE.
42. Carl Bredo Dahl: ANIMAL MODELS IN PSYCHIATRY.

1989

43. Torbjørn A. Fredriksen: CERVICOGENIC HEADACHE.
44. Rolf A. Walstad: CEFTAZIDIME.
45. Rolf Salvesen: THE PUPIL IN CLUSTER HEADACHE.
46. Nils Petter Jørgensen: DRUG EXPOSURE IN EARLY PREGNANCY.
47. Johan C. Ræder: PREMEDICATION AND GENERAL ANAESTHESIA IN OUTPATIENT GYNECOLOGICAL SURGERY.
48. M. R. Shalaby: IMMUNOREGULATORY PROPERTIES OF TNF-α AND THE RELATED CYTOKINES.
49. Anders Waage: THE COMPLEX PATTERN OF CYTOKINES IN SEPTIC SHOCK.
50. Bjarne Christian Eriksen: ELECTROSTIMULATION OF THE PELVIC FLOOR IN FEMALE URINARY INCONTINENCE.
51. Tore B. Halvorsen: PROGNOSTIC FACTORS IN COLORECTAL CANCER.

1990

52. Asbjørn Nordby: CELLULAR TOXICITY OF ROENTGEN CONTRAST MEDIA.
53. Kåre E. Tvedt: X-RAY MICROANALYSIS OF BIOLOGICAL MATERIAL.
54. Tore C. Stiles: COGNITIVE VULNERABILITY FACTORS IN THE DEVELOPMENT AND MAINTENANCE OF DEPRESSION.
55. Eva Hofsli: TUMOR NECROSIS FACTOR AND MULTIDRUG RESISTANCE.
56. Helge S. Haarstad: TROPHIC EFFECTS OF CHOLECYSTOKININ AND SECRETIN ON THE RAT PANCREAS.
57. Lars Engebretsen: TREATMENT OF ACUTE ANTERIOR CRUCIATE LIGAMENT INJURIES.
58. Tarjei Rygnestad: DELIBERATE SELF-POISONING IN TRONDHEIM.
59. Arne Z. Henriksen: STUDIES ON CONSERVED ANTIGENIC DOMAINS ON MAJOR OUTER MEMBRANE PROTEINS FROM ENTEROBACTERIA.
60. Steinar Westin: UNEMPLOYMENT AND HEALTH: Medical and social consequences of a factory closure in a ten-year controlled follow-up study.
61. Ylva Sahlin: INJURY REGISTRATION, a tool for accident preventive work.
62. Helge Bjørnstad Pettersen: BIOSYNTHESIS OF COMPLEMENT BY HUMAN ALVEOLAR MACROPHAGES WITH SPECIAL REFERENCE TO SARCOIDOSIS.
63. Berit Schei: TRAPPED IN PAINFUL LOVE.
64. Lars J. Vatten: PROSPECTIVE STUDIES OF THE RISK OF BREAST CANCER IN A COHORT OF NORWEGIAN WOMAN.

1991

65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.
67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.
68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

1992

74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

1993

82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

1994

92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

1995

104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.
108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

1996

110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANCER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

1997

124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs

1998

132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.

133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.
138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

1999

141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

2000

158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
159. xxxxxxxxx (blind number)
160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.

162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

2001

178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT

192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.

217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA

244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

2006

269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE
271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT

272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS
275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION
279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS
280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
284. Eva Tegnander: DETECTION OF CONGENITAL HEART DEFECTS IN A NON-SELECTED POPULATION OF 42,381 FETUSES
285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OF PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
291. Anne Engum: DEPRESSION AND ANXIETY – THEIR RELATIONS TO THYROID DYSFUNCTION AND DIABETES IN A LARGE EPIDEMIOLOGICAL STUDY
292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
295. Gunilla Klensmeden Fosse: MENTAL HEALTH OF PSYCHIATRIC OUTPATIENTS BULLIED IN CHILDHOOD
296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN

297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

2007

298. Haakon R. Skogseth: INVASIVE PROPERTIES OF CANCER – A TREATMENT TARGET ? IN VITRO STUDIES IN HUMAN PROSTATE CANCER CELL LINES
299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY
300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
302. Siri Malm: LEFT VENTRICULAR SYSTOLIC FUNCTION AND MYOCARDIAL PERFUSION ASSESSED BY CONTRAST ECHOCARDIOGRAPHY
303. Valentina Maria do Rosario Cabral Iversen: MENTAL HEALTH AND PSYCHOLOGICAL ADAPTATION OF CLINICAL AND NON-CLINICAL MIGRANT GROUPS
304. Lasse Løvstakken: SIGNAL PROCESSING IN DIAGNOSTIC ULTRASOUND: ALGORITHMS FOR REAL-TIME ESTIMATION AND VISUALIZATION OF BLOOD FLOW VELOCITY
305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM
306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_2S IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS

324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION
327. Ingebjørg S. Juel : INTESTINAL INJURY AND RECOVERY AFTER ISCHEMIA. AN EXPERIMENTAL STUDY ON RESTITUTION OF THE SURFACE EPITHELIUM, INTESTINAL PERMEABILITY, AND RELEASE OF BIOMARKERS FROM THE MUCOSA
328. Runa Heimstad: POST-TERM PREGNANCY
329. Jan Egil Afset: ROLE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* IN CHILDHOOD DIARRHOEA IN NORWAY
330. Bent Håvard Hellum: *IN VITRO* INTERACTIONS BETWEEN MEDICINAL DRUGS AND HERBS ON CYTOCHROME P-450 METABOLISM AND P-GLYCOPROTEIN TRANSPORT
331. Morten André Høydal: CARDIAC DYSFUNCTION AND MAXIMAL OXYGEN UPTAKE MYOCARDIAL ADAPTATION TO ENDURANCE TRAINING

2008

332. Andreas Møllerløkken: REDUCTION OF VASCULAR BUBBLES: METHODS TO PREVENT THE ADVERSE EFFECTS OF DECOMPRESSION
333. Anne Hege Aamodt: COMORBIDITY OF HEADACHE AND MIGRAINE IN THE NORD-TRØNDELAG HEALTH STUDY 1995-97
334. Brage Høyem Amundsen: MYOCARDIAL FUNCTION QUANTIFIED BY SPECKLE TRACKING AND TISSUE DOPPLER ECHOCARDIOGRAPHY – VALIDATION AND APPLICATION IN EXERCISE TESTING AND TRAINING
335. Inger Anne Næss: INCIDENCE, MORTALITY AND RISK FACTORS OF FIRST VENOUS THROMBOSIS IN A GENERAL POPULATION. RESULTS FROM THE SECOND NORD-TRØNDELAG HEALTH STUDY (HUNT2)
336. Vegard Bugten: EFFECTS OF POSTOPERATIVE MEASURES AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY
337. Morten Bruvold: MANGANESE AND WATER IN CARDIAC MAGNETIC RESONANCE IMAGING
338. Miroslav Fris: THE EFFECT OF SINGLE AND REPEATED ULTRAVIOLET RADIATION ON THE ANTERIOR SEGMENT OF THE RABBIT EYE
339. Svein Arne Aase: METHODS FOR IMPROVING QUALITY AND EFFICIENCY IN QUANTITATIVE ECHOCARDIOGRAPHY – ASPECTS OF USING HIGH FRAME RATE
340. Roger Almvik: ASSESSING THE RISK OF VIOLENCE: DEVELOPMENT AND VALIDATION OF THE BRØSET VIOLENCE CHECKLIST
341. Ottar Sundheim: STRUCTURE-FUNCTION ANALYSIS OF HUMAN ENZYMES INITIATING NUCLEOBASE REPAIR IN DNA AND RNA
342. Anne Mari Undheim: SHORT AND LONG-TERM OUTCOME OF EMOTIONAL AND BEHAVIOURAL PROBLEMS IN YOUNG ADOLESCENTS WITH AND WITHOUT READING DIFFICULTIES
343. Helge Garåsen: THE TRONDHEIM MODEL. IMPROVING THE PROFESSIONAL COMMUNICATION BETWEEN THE VARIOUS LEVELS OF HEALTH CARE SERVICES AND IMPLEMENTATION OF INTERMEDIATE CARE AT A COMMUNITY HOSPITAL COULD PROVIDE BETTER CARE FOR OLDER PATIENTS. SHORT AND LONG TERM EFFECTS
344. Olav A. Foss: "THE ROTATION RATIOS METHOD". A METHOD TO DESCRIBE ALTERED SPATIAL ORIENTATION IN SEQUENTIAL RADIOGRAPHS FROM ONE PELVIS
345. Bjørn Olav Åsvold: THYROID FUNCTION AND CARDIOVASCULAR HEALTH
346. Torun Margareta Melø: NEURONAL GLIAL INTERACTIONS IN EPILEPSY
347. Irina Poliakova Eide: FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA: SOME CHARACTERISTICS OF FETO-MATERNAL INTERACTIONS IN DECIDUA BASALIS
348. Torunn Askim: RECOVERY AFTER STROKE. ASSESSMENT AND TREATMENT; WITH FOCUS ON MOTOR FUNCTION
349. Ann Elisabeth Åsberg: NEUTROPHIL ACTIVATION IN A ROLLER PUMP MODEL OF CARDIOPULMONARY BYPASS. INFLUENCE ON BIOMATERIAL, PLATELETS AND COMPLEMENT

350. Lars Hagen: REGULATION OF DNA BASE EXCISION REPAIR BY PROTEIN INTERACTIONS AND POST TRANSLATIONAL MODIFICATIONS
351. Sigrun Beate Kjøtrød: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN ASSISTED REPRODUCTION
352. Steven Keita Nishiyama: PERSPECTIVES ON LIMB-VASCULAR HETEROGENEITY: IMPLICATIONS FOR HUMAN AGING, SEX, AND EXERCISE
353. Sven Peter Näsholm: ULTRASOUND BEAMS FOR ENHANCED IMAGE QUALITY
354. Jon Ståle Ritland: PRIMARY OPEN-ANGLE GLAUCOMA & EXFOLIATIVE GLAUCOMA. SURVIVAL, COMORBIDITY AND GENETICS
355. Sigrid Botne Sando: ALZHEIMER'S DISEASE IN CENTRAL NORWAY. GENETIC AND EDUCATIONAL ASPECTS
356. Parvinder Kaur: CELLULAR AND MOLECULAR MECHANISMS BEHIND METHYLMERCURY-INDUCED NEUROTOXICITY
357. Ismail Cüneyt Güzey: DOPAMINE AND SEROTONIN RECEPTOR AND TRANSPORTER GENE POLYMORPHISMS AND EXTRAPYRAMIDAL SYMPTOMS. STUDIES IN PARKINSON'S DISEASE AND IN PATIENTS TREATED WITH ANTIPSYCHOTIC OR ANTIDEPRESSANT DRUGS
358. Brit Dybdahl: EXTRA-CELLULAR INDUCIBLE HEAT-SHOCK PROTEIN 70 (Hsp70) – A ROLE IN THE INFLAMMATORY RESPONSE ?
359. Kristoffer Haugarvoll: IDENTIFYING GENETIC CAUSES OF PARKINSON'S DISEASE IN NORWAY
360. Nadra Nilsen: TOLL-LIKE RECEPTOR 2 –EXPRESSION, REGULATION AND SIGNALING
361. Johan Håkon Bjørngaard: PATIENT SATISFACTION WITH OUTPATIENT MENTAL HEALTH SERVICES – THE INFLUENCE OF ORGANIZATIONAL FACTORS.
362. Kjetil Høydal : EFFECTS OF HIGH INTENSITY AEROBIC TRAINING IN HEALTHY SUBJECTS AND CORONARY ARTERY DISEASE PATIENTS; THE IMPORTANCE OF INTENSITY,, DURATION AND FREQUENCY OF TRAINING.
363. Trine Karlsen: TRAINING IS MEDICINE: ENDURANCE AND STRENGTH TRAINING IN CORONARY ARTERY DISEASE AND HEALTH.
364. Marte Thuen: MANGANASE-ENHANCED AND DIFFUSION TENSOR MR IMAGING OF THE NORMAL, INJURED AND REGENERATING RAT VISUAL PATHWAY
365. Cathrine Broberg Vågbø: DIRECT REPAIR OF ALKYLATION DAMAGE IN DNA AND RNA BY 2-OXOGLUTARATE- AND IRON-DEPENDENT DIOXYGENASES
366. Arnt Erik Tjønna: AEROBIC EXERCISE AND CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT AND OBESE ADOLESCENTS AND ADULTS
367. Marianne W. Furnes: FEEDING BEHAVIOR AND BODY WEIGHT DEVELOPMENT: LESSONS FROM RATS
368. Lene N. Johannessen: FUNGAL PRODUCTS AND INFLAMMATORY RESPONSES IN HUMAN MONOCYTES AND EPITHELIAL CELLS
369. Anja Bye: GENE EXPRESSION PROFILING OF *INHERITED* AND *ACQUIRED* MAXIMAL OXYGEN UPTAKE – RELATIONS TO THE METABOLIC SYNDROME.
370. Oluf Dimitri Røe: MALIGNANT MESOTHELIOMA: VIRUS, BIOMARKERS AND GENES. A TRANSLATIONAL APPROACH
371. Ane Cecilie Dale: DIABETES MELLITUS AND FATAL ISCHEMIC HEART DISEASE. ANALYSES FROM THE HUNT1 AND 2 STUDIES
372. Jacob Christian Hølen: PAIN ASSESSMENT IN PALLIATIVE CARE: VALIDATION OF METHODS FOR SELF-REPORT AND BEHAVIOURAL ASSESSMENT
373. Erming Tian: THE GENETIC IMPACTS IN THE ONCOGENESIS OF MULTIPLE MYELOMA
374. Ole Bosnes: KLINISK UTPRØVING AV NORSKE VERSJONER AV NOEN SENTRALE TESTER PÅ KOGNITIV FUNKSJON
375. Ola M. Rygh: 3D ULTRASOUND BASED NEURONAVIGATION IN NEUROSURGERY. A CLINICAL EVALUATION
376. Astrid Kamilla Stunes: ADIPOKINES, PEROXISOME PROFILERATOR ACTIVATED RECEPTOR (PPAR) AGONISTS AND SEROTONIN. COMMON REGULATORS OF BONE AND FAT METABOLISM
377. Silje Engdal: HERBAL REMEDIES USED BY NORWEGIAN CANCER PATIENTS AND THEIR ROLE IN HERB-DRUG INTERACTIONS
378. Kristin Offerdal: IMPROVED ULTRASOUND IMAGING OF THE FETUS AND ITS CONSEQUENCES FOR SEVERE AND LESS SEVERE ANOMALIES

379. Øivind Rognmo: HIGH-INTENSITY AEROBIC EXERCISE AND CARDIOVASCULAR HEALTH
380. Jo-Åsmund Lund: RADIOTHERAPY IN ANAL CARCINOMA AND PROSTATE CANCER

2009

381. Tore Grüner Bjåstad: HIGH FRAME RATE ULTRASOUND IMAGING USING PARALLEL BEAMFORMING
382. Erik Søndenaa: INTELLECTUAL DISABILITIES IN THE CRIMINAL JUSTICE SYSTEM
383. Berit Rostad: SOCIAL INEQUALITIES IN WOMEN'S HEALTH, HUNT 1984-86 AND 1995-97, THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
384. Jonas Crosby: ULTRASOUND-BASED QUANTIFICATION OF MYOCARDIAL DEFORMATION AND ROTATION
385. Erling Tronvik: MIGRAINE, BLOOD PRESSURE AND THE RENIN-ANGIOTENSIN SYSTEM
386. Tom Christensen: BRINGING THE GP TO THE FOREFRONT OF EPR DEVELOPMENT
387. Håkon Bergseng: ASPECTS OF GROUP B STREPTOCOCCUS (GBS) DISEASE IN THE NEWBORN. EPIDEMIOLOGY, CHARACTERISATION OF INVASIVE STRAINS AND EVALUATION OF INTRAPARTUM SCREENING
388. Ronny Myhre: GENETIC STUDIES OF CANDIDATE TENE3S IN PARKINSON'S DISEASE
389. Torbjørn Moe Eggebø: ULTRASOUND AND LABOUR
390. Eivind Wang: TRAINING IS MEDICINE FOR PATIENTS WITH PERIPHERAL ARTERIAL DISEASE
391. Thea Kristin Våtsveen: GENETIC ABERRATIONS IN MYELOMA CELLS
392. Thomas Jozefiak: QUALITY OF LIFE AND MENTAL HEALTH IN CHILDREN AND ADOLESCENTS: CHILD AND PARENT PERSPECTIVES
393. Jens Erik Slagsvold: N-3 POLYUNSATURATED FATTY ACIDS IN HEALTH AND DISEASE – CLINICAL AND MOLECULAR ASPECTS
394. Kristine Misund: A STUDY OF THE TRANSCRIPTIONAL REPRESSOR ICER. REGULATORY NETWORKS IN GASTRIN-INDUCED GENE EXPRESSION
395. Franco M. Impellizzeri: HIGH-INTENSITY TRAINING IN FOOTBALL PLAYERS. EFFECTS ON PHYSICAL AND TECHNICAL PERFORMANCE
396. Kari Hanne Gjeilo: HEALTH-RELATED QUALITY OF LIFE AND CHRONIC PAIN IN PATIENTS UNDERGOING CARDIAC SURGERY
397. Øyvind Hauso: NEUROENDOCRINE ASPECTS OF PHYSIOLOGY AND DISEASE
398. Ingvild Bjellmo Johnsen: INTRACELLULAR SIGNALING MECHANISMS IN THE INNATE IMMUNE RESPONSE TO VIRAL INFECTIONS
399. Linda Tømmerdal Roten: GENETIC PREDISPOSITION FOR DEVELOPMENT OF PREEMCLAMPSIA – CANDIDATE GENE STUDIES IN THE HUNT (NORD-TRØNDELAG HEALTH STUDY) POPULATION
400. Trude Teoline Nausthaug Rakvåg: PHARMACOGENETICS OF MORPHINE IN CANCER PAIN
401. Hanne Lehn: MEMORY FUNCTIONS OF THE HUMAN MEDIAL TEMPORAL LOBE STUDIED WITH fMRI
402. Randi Utne Holt: ADHESION AND MIGRATION OF MYELOMA CELLS – IN VITRO STUDIES –
403. Trygve Solstad: NEURAL REPRESENTATIONS OF EUCLIDEAN SPACE
404. Unn-Merete Fagerli: MULTIPLE MYELOMA CELLS AND CYTOKINES FROM THE BONE MARROW ENVIRONMENT; ASPECTS OF GROWTH REGULATION AND MIGRATION
405. Sigrid Bjørnelv: EATING– AND WEIGHT PROBLEMS IN ADOLESCENTS, THE YOUNG HUNT-STUDY
406. Mari Hoff: CORTICAL HAND BONE LOSS IN RHEUMATOID ARTHRITIS. EVALUATING DIGITAL X-RAY RADIOGRAMMETRY AS OUTCOME MEASURE OF DISEASE ACTIVITY, RESPONSE VARIABLE TO TREATMENT AND PREDICTOR OF BONE DAMAGE
407. Siri Bjørgen: AEROBIC HIGH INTENSITY INTERVAL TRAINING IS AN EFFECTIVE TREATMENT FOR PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE
408. Susanne Lindqvist: VISION AND BRAIN IN ADOLESCENTS WITH LOW BIRTH WEIGHT
409. Torbjørn Hergum: 3D ULTRASOUND FOR QUANTITATIVE ECHOCARDIOGRAPHY

410. Jørgen Urnes: PATIENT EDUCATION IN GASTRO-OESOPHAGEAL REFLUX DISEASE. VALIDATION OF A DIGESTIVE SYMPTOMS AND IMPACT QUESTIONNAIRE AND A RANDOMISED CONTROLLED TRIAL OF PATIENT EDUCATION
411. Elvar Eyjolfsson: 13C NMRS OF ANIMAL MODELS OF SCHIZOPHRENIA
412. Marius Steiro Fimland: CHRONIC AND ACUTE NEURAL ADAPTATIONS TO STRENGTH TRAINING
413. Øyvind Støren: RUNNING AND CYCLING ECONOMY IN ATHLETES; DETERMINING FACTORS, TRAINING INTERVENTIONS AND TESTING
414. Håkon Hov: HEPATOCYTE GROWTH FACTOR AND ITS RECEPTOR C-MET. AUTOCRINE GROWTH AND SIGNALING IN MULTIPLE MYELOMA CELLS
415. Maria Radtke: ROLE OF AUTOIMMUNITY AND OVERSTIMULATION FOR BETA-CELL DEFICIENCY. EPIDEMIOLOGICAL AND THERAPEUTIC PERSPECTIVES
416. Liv Bente Romundstad: ASSISTED FERTILIZATION IN NORWAY: SAFETY OF THE REPRODUCTIVE TECHNOLOGY
417. Erik Magnus Berntsen: PREOPERATIV PLANNING AND FUNCTIONAL NEURONAVIGATION – WITH FUNCTIONAL MRI AND DIFFUSION TENSOR TRACTOGRAPHY IN PATIENTS WITH BRAIN LESIONS
418. Tonje Strømmen Steigedal: MOLECULAR MECHANISMS OF THE PROLIFERATIVE RESPONSE TO THE HORMONE GASTRIN
419. Vidar Rao: EXTRACORPOREAL PHOTOCHEMOTHERAPY IN PATIENTS WITH CUTANEOUS T CELL LYMPHOMA OR GRAFT-vs-HOST DISEASE
420. Torkild Visnes: DNA EXCISION REPAIR OF URACIL AND 5-FLUOROURACIL IN HUMAN CANCER CELL LINES

2010

421. John Munkhaugen: BLOOD PRESSURE, BODY WEIGHT, AND KIDNEY FUNCTION IN THE NEAR-NORMAL RANGE: NORMALITY, RISK FACTOR OR MORBIDITY ?
422. Ingrid Castberg: PHARMACOKINETICS, DRUG INTERACTIONS AND ADHERENCE TO TREATMENT WITH ANTIPSYCHOTICS: STUDIES IN A NATURALISTIC SETTING
423. Jian Xu: BLOOD-OXYGEN-LEVEL-DEPENDENT-FUNCTIONAL MAGNETIC RESONANCE IMAGING AND DIFFUSION TENSOR IMAGING IN TRAUMATIC BRAIN INJURY RESEARCH
424. Sigmund Simonsen: ACCEPTABLE RISK AND THE REQUIREMENT OF PROPORTIONALITY IN EUROPEAN BIOMEDICAL RESEARCH LAW. WHAT DOES THE REQUIREMENT THAT BIOMEDICAL RESEARCH SHALL NOT INVOLVE RISKS AND BURDENS DISPROPORTIONATE TO ITS POTENTIAL BENEFITS MEAN?
425. Astrid Woodhouse: MOTOR CONTROL IN WHIPLASH AND CHRONIC NON-TRAUMATIC NECK PAIN
426. Line Rørstad Jensen: EVALUATION OF TREATMENT EFFECTS IN CANCER BY MR IMAGING AND SPECTROSCOPY
427. Trine Moholdt: AEROBIC EXERCISE IN CORONARY HEART DISEASE
428. Øystein Olsen: ANALYSIS OF MANGANESE ENHANCED MRI OF THE NORMAL AND INJURED RAT CENTRAL NERVOUS SYSTEM
429. Bjørn H. Grønberg: PEMETREXED IN THE TREATMENT OF ADVANCED LUNG CANCER
430. Vigdis Schnell Husby: REHABILITATION OF PATIENTS UNDERGOING TOTAL HIP ARTHROPLASTY WITH FOCUS ON MUSCLE STRENGTH, WALKING AND AEROBIC ENDURANCE PERFORMANCE
431. Torbjørn Øien: CHALLENGES IN PRIMARY PREVENTION OF ALLERGY. THE PREVENTION OF ALLERGY AMONG CHILDREN IN TRONDHEIM (PACT) STUDY.
432. Kari Anne Indredavik Evensen: BORN TOO SOON OR TOO SMALL: MOTOR PROBLEMS IN ADOLESCENCE
433. Lars Adde: PREDICTION OF CEREBRAL PALSY IN YOUNG INFANTS. COMPUTER BASED ASSESSMENT OF GENERAL MOVEMENTS
434. Magnus Fasting: PRE- AND POSTNATAL RISK FACTORS FOR CHILDHOOD ADIPOSITY
435. Vivi Talstad Monsen: MECHANISMS OF ALKYLATION DAMAGE REPAIR BY HUMAN AlkB HOMOLOGUES
436. Toril Skandsen: MODERATE AND SEVERE TRAUMATIC BRAIN INJURY. MAGNETIC RESONANCE IMAGING FINDINGS, COGNITION AND RISK FACTORS FOR DISABILITY

437. Ingeborg Smidesang: ALLERGY RELATED DISORDERS AMONG 2-YEAR OLDS AND ADOLESCENTS IN MID-NORWAY – PREVALENCE, SEVERITY AND IMPACT. THE PACT STUDY 2005, THE YOUNG HUNT STUDY 1995-97
438. Vidar Halsteinli: MEASURING EFFICIENCY IN MENTAL HEALTH SERVICE DELIVERY: A STUDY OF OUTPATIENT UNITS IN NORWAY
439. Karen Lehrmann Ægidius: THE PREVALENCE OF HEADACHE AND MIGRAINE IN RELATION TO SEX HORMONE STATUS IN WOMEN. THE HUNT 2 STUDY
440. Madelene Ericsson: EXERCISE TRAINING IN GENETIC MODELS OF HEART FAILURE
441. Marianne Klokk: THE ASSOCIATION BETWEEN SELF-REPORTED ECZEMA AND COMMON MENTAL DISORDERS IN THE GENERAL POPULATION. THE HORDALAND HEALTH STUDY (HUSK)
442. Tomas Ottemo Stølen: IMPAIRED CALCIUM HANDLING IN ANIMAL AND HUMAN CARDIOMYOCYTES REDUCE CONTRACTILITY AND INCREASE ARRHYTHMIA POTENTIAL – EFFECTS OF AEROBIC EXERCISE TRAINING
443. Bjarne Hansen: ENHANCING TREATMENT OUTCOME IN COGNITIVE BEHAVIOURAL THERAPY FOR OBSESSIVE COMPULSIVE DISORDER: THE IMPORTANCE OF COGNITIVE FACTORS
444. Mona Løvlien: WHEN EVERY MINUTE COUNTS. FROM SYMPTOMS TO ADMISSION FOR ACUTE MYOCARDIAL INFARCTION WITH SPECIAL EMPHASIS ON GENDER DIFFERECES
445. Karin Margaretha Gilljam: DNA REPAIR PROTEIN COMPLEXES, FUNCTIONALITY AND SIGNIFICANCE FOR REPAIR EFFICIENCY AND CELL SURVIVAL
446. Anne Byriel Walls: NEURONAL GLIAL INTERACTIONS IN CEREBRAL ENERGY – AND AMINO ACID HOMEOSTASIS – IMPLICATIONS OF GLUTAMATE AND GABA
447. Cathrine Fallang Knetter: MECHANISMS OF TOLL-LIKE RECEPTOR 9 ACTIVATION
448. Marit Følsvik Svindseth: A STUDY OF HUMILIATION, NARCISSISM AND TREATMENT OUTCOME IN PATIENTS ADMITTED TO PSYCHIATRIC EMERGENCY UNITS
449. Karin Elvenes Bakkelund: GASTRIC NEUROENDOCRINE CELLS – ROLE IN GASTRIC NEOPLASIA IN MAN AND RODENTS
450. Kirsten Brun Kjelstrup: DORSOVENTRAL DIFFERENCES IN THE SPATIAL REPRESENTATION AREAS OF THE RAT BRAIN
451. Roar Johansen: MR EVALUATION OF BREAST CANCER PATIENTS WITH POOR PROGNOSIS
452. Rigmor Myran: POST TRAUMATIC NECK PAIN. EPIDEMIOLOGICAL, NEURORADIOLOGICAL AND CLINICAL ASPECTS
453. Krisztina Kunszt Johansen: GENEALOGICAL, CLINICAL AND BIOCHEMICAL STUDIES IN *LRRK2* – ASSOCIATED PARKINSON'S DISEASE
454. Pål Gjerden: THE USE OF ANTICHOLINERGIC ANTIPARKINSON AGENTS IN NORWAY. EPIDEMIOLOGY, TOXICOLOGY AND CLINICAL IMPLICATIONS
455. Else Marie Huuse: ASSESSMENT OF TUMOR MICROENVIRONMENT AND TREATMENT EFFECTS IN HUMAN BREAST CANCER XENOGRAFTS USING MR IMAGING AND SPECTROSCOPY
456. Khalid S. Ibrahim: INTRAOPERATIVE ULTRASOUND ASSESSMENT IN CORONARY ARTERY BYPASS SURGERY – WITH SPECIAL REFERENCE TO CORONARY ANASTOMOSES AND THE ASCENDING AORTA
457. Bjørn Øglænd: ANTHROPOMETRY, BLOOD PRESSURE AND REPRODUCTIVE DEVELOPMENT IN ADOLESCENCE OF OFFSPRING OF MOTHERS WHO HAD PREECLAMPSIA IN PREGNANCY
458. John Olav Roaldset: RISK ASSESSMENT OF VIOLENT, SUICIDAL AND SELF-INJURIOUS BEHAVIOUR IN ACUTE PSYCHIATRY – A BIO-PSYCHO-SOCIAL APPROACH
459. Håvard Dalen: ECHOCARDIOGRAPHIC INDICES OF CARDIAC FUNCTION – NORMAL VALUES AND ASSOCIATIONS WITH CARDIAC RISK FACTORS IN A POPULATION FREE FROM CARDIOVASCULAR DISEASE, HYPERTENSION AND DIABETES: THE HUNT 3 STUDY
460. Beate André: CHANGE CAN BE CHALLENGING. INTRODUCTION TO CHANGES AND IMPLEMENTATION OF COMPUTERIZED TECHNOLOGY IN HEALTH CARE
461. Latha Nrugham: ASSOCIATES AND PREDICTORS OF ATTEMPTED SUICIDE AMONG DEPRESSED ADOLESCENTS – A 6-YEAR PROSPECTIVE STUDY

462. Håvard Bersås Nordgaard: TRANSIT-TIME FLOWMETRY AND WALL SHEAR STRESS ANALYSIS OF CORONARY ARTERY BYPASS GRAFTS – A CLINICAL AND EXPERIMENTAL STUDY

Cotutelle with University of Ghent: Abigail Emily Swillens: A MULTIPHYSICS MODEL FOR IMPROVING THE ULTRASONIC ASSESSMENT OF LARGE ARTERIES

2011

463. Marte Helene Bjørk: DO BRAIN RHYTHMS CHANGE BEFORE THE MIGRAINE ATTACK? A LONGITUDINAL CONTROLLED EEG STUDY
464. Carl-Jørgen Arum: A STUDY OF UROTHELIAL CARCINOMA: GENE EXPRESSION PROFILING, TUMORIGENESIS AND THERAPIES IN ORTHOTOPIC ANIMAL MODELS
465. Ingunn Harstad: TUBERCULOSIS INFECTION AND DISEASE AMONG ASYLUM SEEKERS IN NORWAY. SCREENING AND FOLLOW-UP IN PUBLIC HEALTH CARE
466. Leif Åge Strand: EPIDEMIOLOGICAL STUDIES AMONG ROYAL NORWEGIAN NAVY SERVICEMEN. COHORT ESTABLISHMENT, CANCER INCIDENCE AND CAUSE-SPECIFIC MORTALITY
467. Katrine Høyer Holgersen: SURVIVORS IN THEIR THIRD DECADE AFTER THE NORTH SEA OIL RIG DISASTER OF 1980. LONG-TERM PERSPECTIVES ON MENTAL HEALTH
468. MarianneWallenius: PREGNANCY RELATED ASPECTS OF CHRONIC INFLAMMATORY ARTHRITIDES: DISEASE ONSET POSTPARTUM, PREGNANCY OUTCOMES AND FERTILITY. DATA FROM A NORWEGIAN PATIENT REGISTRY LINKED TO THE MEDICAL BIRTH REGISTRY OF NORWAY
469. Ole Vegard Solberg: 3D ULTRASOUND AND NAVIGATION – APPLICATIONS IN LAPAROSCOPIC SURGERY
470. Inga Ekeberg Schjerve: EXERCISE-INDUCED IMPROVEMENT OF MAXIMAL OXYGEN UPTAKE AND ENDOTHELIAL FUNCTION IN OBESE AND OVERWEIGHT INDIVIDUALS ARE DEPENDENT ON EXERCISE-INTENSITY
471. Eva Veslemøy Tyldum: CARDIOVASCULAR FUNCTION IN PREECLAMPSIA – WITH REFERENCE TO ENDOTHELIAL FUNCTION, LEFT VENTRICULAR FUNCTION AND PRE-PREGNANCY PHYSICAL ACTIVITY
472. Benjamin Garzón Jiménez de Cisneros: CLINICAL APPLICATIONS OF MULTIMODAL MAGNETIC RESONANCE IMAGING
473. Halvard Knut Nilsen: ASSESSING CODEINE TREATMENT TO PATIENTS WITH CHRONIC NON-MALIGNANT PAIN: NEUROPSYCHOLOGICAL FUNCTIONING, DRIVING ABILITY AND WEANING
474. Eiliv Brenner: GLUTAMATE RELATED METABOLISM IN ANIMAL MODELS OF SCHIZOPHRENIA
475. Egil Jonsbu: CHEST PAIN AND PALPITATIONS IN A CARDIAC SETTING; PSYCHOLOGICAL FACTORS, OUTCOME AND TREATMENT
476. Mona Høysæter Fenstad: GENETIC SUSCEPTIBILITY TO PREECLAMPSIA : STUDIES ON THE NORD-TRØNDELAG HEALTH STUDY (HUNT) COHORT, AN AUSTRALIAN/NEW ZEALAND FAMILY COHORT AND DECIDUA BASALIS TISSUE
477. Svein Erik Gaustad: CARDIOVASCULAR CHANGES IN DIVING: FROM HUMAN RESPONSE TO CELL FUNCTION
478. Karin Torvik: PAIN AND QUALITY OF LIFE IN PATIENTS LIVING IN NURSING HOMES
479. Arne Solberg: OUTCOME ASSESSMENTS IN NON-METASTATIC PROSTATE CANCER
480. Henrik Sahlin Pettersen: CYTOTOXICITY AND REPAIR OF URACIL AND 5-FLUOROURACIL IN DNA
481. Pui-Lam Wong: PHYSICAL AND PHYSIOLOGICAL CAPACITY OF SOCCER PLAYERS: EFFECTS OF STRENGTH AND CONDITIONING
482. Ole Solheim: ULTRASOUND GUIDED SURGERY IN PATIENTS WITH INTRACRANIAL TUMOURS
483. Sten Roar Snare: QUANTITATIVE CARDIAC ANALYSIS ALGORITHMS FOR POCKET-SIZED ULTRASOUND DEVICES
484. Marit Skyrud Bratlie: LARGE-SCALE ANALYSIS OF ORTHOLOGS AND PARALOGS IN VIRUSES AND PROKARYOTES
485. Anne Elisabeth F. Isern: BREAST RECONSTRUCTION AFTER MASTECTOMY – RISK OF RECURRENCE AFTER DELAYED LARGE FLAP RECONSTRUCTION – AESTHETIC OUTCOME, PATIENT SATISFACTION, QUALITY OF LIFE AND SURGICAL RESULTS;

HISTOPATHOLOGICAL FINDINGS AND FOLLOW-UP AFTER PROPHYLACTIC MASTECTOMY IN HEREDITARY BREAST CANCER

486. Guro L. Andersen: CEREBRAL PALSY IN NORWAY – SUBTYPES, SEVERITY AND RISK FACTORS
487. Frode Kolstad: CERVICAL DISC DISEASE – BIOMECHANICAL ASPECTS
488. Bente Nordtug: CARING BURDEN OF COHABITANTS LIVING WITH PARTNERS SUFFERING FROM CHRONIC OBSTRUCTIVE PULMONARY DISEASE OR DEMENTIA
489. Mariann Gjervik Heldahl: EVALUATION OF NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER BASED ON MR METHODOLOGY
490. Lise Tevik Løvseth: THE SUBJECTIVE BURDEN OF CONFIDENTIALITY
491. Marie Hjelmseth Aune: INFLAMMATORY RESPONSES AGAINST GRAM NEGATIVE BACTERIA INDUCED BY TLR4 AND NLRP12
492. Tina Strømdal Wik: EXPERIMENTAL EVALUATION OF NEW CONCEPTS IN HIP ARTHROPLASTY
493. Solveig Sigurdardottir: CLINICAL ASPECTS OF CEREBRAL PALSY IN ICELAND. A POPULATION-BASED STUDY OF PRESCHOOL CHILDREN
494. Arne Reimers: CLINICAL PHARMACOKINETICS OF LAMOTRIGINE
495. Monica Wegling: KULTURMENNESKETS BYRDE OG SYKDOMMENS VELSIGNELSE. KAN MEDISINSK UTREDNING OG INTERVENSJON HA EN SELVSTENDIG FUNKSJON UAVHENGIG AV DET KURATIVE?
496. Silje Alvestad: ASTROCYTE-NEURON INTERACTIONS IN EXPERIMENTAL MESIAL TEMPORAL LOBE EPILEPSY – A STUDY OF UNDERLYING MECHANISMS AND POSSIBLE BIOMARKERS OF EPILEPTOGENESIS
497. Javaid Nauman: RESTING HEART RATE: A MATTER OF LIFE OR DEATH – PROSPECTIVE STUDIES OF RESTING HEART RATE AND CARDIOVASCULAR RISK (THE HUNT STUDY, NORWAY)
498. Thuy Nguyen: THE ROLE OF C-SRC TYROSINE KINASE IN ANTIVIRAL IMMUNE RESPONSES
499. Trine Naalsund Andreassen: PHARMACOKINETIC, PHARMACODYNAMIC AND PHARMACOGENETIC ASPECTS OF OXYCODONE TREATMENT IN CANCER PAIN
500. Eivor Alette Laugsand: SYMPTOMS IN PATIENTS RECEIVING OPIOIDS FOR CANCER PAIN – CLINICAL AND PHARMACOGENETIC ASPECTS
501. Dorthe Stensvold: PHYSICAL ACTIVITY, CARDIOVASCULAR HEALTH AND LONGEVITY IN PATIENTS WITH METABOLIC SYNDROME
502. Stian Thoresen Aspenes: PEAK OXYGEN UPTAKE AMONG HEALTHY ADULTS – CROSS-SECTIONAL DESCRIPTIONS AND PROSPECTIVE ANALYSES OF PEAK OXYGEN UPTAKE, PHYSICAL ACTIVITY AND CARDIOVASCULAR RISK FACTORS IN HEALTHY ADULTS (20-90 YEARS)
503. Reidar Alexander Vigen: PATHOBIOLOGY OF GASTRIC CARCINOIDS AND ADENOCARCINOMAS IN RODENT MODELS AND PATIENTS. STUDIES OF GASTROCYSTOPLASTY, GENDER-RELATED FACTORS, AND AUTOPHAGY
504. Halvard Høilund-Kaupang: MODELS AND METHODS FOR INVESTIGATION OF REVERBERATIONS IN NONLINEAR ULTRASOUND IMAGING
505. Audhild Løhre: WELLBEING AMONG SCHOOL CHILDREN IN GRADES 1-10: PROMOTING AND ADVERSE FACTORS
506. Torgrim Tandstad: VOX POPULI. POPULATION-BASED OUTCOME STUDIES IN TESTICULAR CANCER
507. Anna Brenne Grønskag: THE EPIDEMIOLOGY OF HIP FRACTURES AMONG ELDERLY WOMEN IN NORD-TRØNDELAG. HUNT 1995-97, THE NORD-TRØNDELAG HEALTH STUDY
508. Kari Ravndal Risnes: BIRTH SIZE AND ADULT MORTALITY: A SYSTEMATIC REVIEW AND A LONG-TERM FOLLOW-UP OF NEARLY 40 000 INDIVIDUALS BORN AT ST. OLAV UNIVERSITY HOSPITAL IN TRONDHEIM 1920-1960
509. Hans Jakob Bøe: LONG-TERM POSTTRAUMATIC STRESS AFTER DISASTER – A CONTROLLED STUDY OF SURVIVORS' HEALTH 27 YEARS AFTER THE CAPSIZED NORTH SEA OIL RIG
510. Cathrin Barbara Canto, Cotutelle with University of Amsterdam: LAYER SPECIFIC INTEGRATIVE PROPERTIES OF ENTORHINAL PRINCIPAL NEURONS
511. Ioanna Sandvig: THE ROLE OF OLFACTORY ENSHEATHING CELLS, MRI, AND BIOMATERIALS IN TRANSPLANT-MEDIATED CNS REPAIR

512. Karin Fahl Wader: HEPATOCYTE GROWTH FACTOR, C-MET AND SYNDECAN-1 IN MULTIPLE MYELOMA
513. Gerd Tranø: FAMILIAL COLORECTAL CANCER
514. Bjarte Bergstrøm: INNATE ANTIVIRAL IMMUNITY – MECHANISMS OF THE RIG-I-MEDIATED RESPONSE
515. Marie Søfteland Sandvei: INCIDENCE, MORTALITY, AND RISK FACTORS FOR ANEURYSMAL SUBARACHNOID HEMORRHAGE. PROSPECTIVE ANALYZES OF THE HUNT AND TROMSØ STUDIES
516. Mary-Elizabeth Bradley Eilertsen: CHILDREN AND ADOLESCENTS SURVIVING CANCER: PSYCHOSOCIAL HEALTH, QUALITY OF LIFE AND SOCIAL SUPPORT
517. Takaya Saito: COMPUTATIONAL ANALYSIS OF REGULATORY MECHANISM AND INTERACTIONS OF MICRORNAS

Godkjent for disputas, publisert post mortem: Eivind Jullumstrø: COLORECTAL CANCER AT LEVANGER HOSPITAL 1980-2004

518. Christian Gutvik: A PHYSIOLOGICAL APPROACH TO A NEW DECOMPRESSION ALGORITHM USING NONLINEAR MODEL PREDICTIVE CONTROL
519. Ola Storrø: MODIFICATION OF ADJUVANT RISK FACTOR BEHAVIOURS FOR ALLERGIC DISEASE AND ASSOCIATION BETWEEN EARLY GUT MICROBIOTA AND ATOPIC SENSITIZATION AND ECZEMA. EARLY LIFE EVENTS DEFINING THE FUTURE HEALTH OF OUR CHILDREN
520. Guro Fanneløb Giskeødegård: IDENTIFICATION AND CHARACTERIZATION OF PROGNOSTIC FACTORS IN BREAST CANCER USING MR METABOLOMICS
521. Gro Christine Christensen Løhaugen: BORN PRETERM WITH VERY LOW BIRTH WEIGHT – NEVER ENDING COGNITIVE CONSEQUENCES?
522. Sigrid Nakrem: MEASURING QUALITY OF CARE IN NURSING HOMES – WHAT MATTERS?
523. Brita Pukstad: CHARACTERIZATION OF INNATE INFLAMMATORY RESPONSES IN ACUTE AND CHRONIC WOUNDS

2012

524. Hans H. Wasmuth: ILEAL POUCHES
525. Inger Økland: BIASES IN SECOND-TRIMESTER ULTRASOUND DATING RELATED TO PREDICTION MODELS AND FETAL MEASUREMENTS
526. Bjørn Mørkedal: BLOOD PRESSURE, OBESITY, SERUM IRON AND LIPIDS AS RISK FACTORS OF ISCHAEMIC HEART DISEASE
527. Siver Andreas Moestue: MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF BREAST CANCER THROUGH A COMBINATION OF MR IMAGING, TRANSCRIPTOMICS AND METABOLOMICS
528. Guro Aune: CLINICAL, PATHOLOGICAL, AND MOLECULAR CLASSIFICATION OF OVARIAN CARCINOMA
529. Ingrid Alsos Lian: MECHANISMS INVOLVED IN THE PATHOGENESIS OF PRE-ECLAMPSIA AND FETAL GROWTH RESTRICTION. TRANSCRIPTIONAL ANALYSES OF PLACENTAL AND DECIDUAL TISSUE
530. Karin Solvang-Garten: X-RAY REPAIR CROSS-COMPLEMENTING PROTEIN 1 – THE ROLE AS A SCAFFOLD PROTEIN IN BASE EXCISION REPAIR AND SINGLE STRAND BREAK REPAIR
531. Toril Holien: BONE MORPHOGENETIC PROTEINS AND MYC IN MULTIPLE MYELOMA
532. Rooyen Mavenyengwa: *STREPTOCOCCUS AGALACTIAE* IN PREGNANT WOMEN IN ZIMBABWE: EPIDEMIOLOGY AND SEROTYPE MARKER CHARACTERISTICS
533. Tormod Rimehaug: EMOTIONAL DISTRESS AND PARENTING AMONG COMMUNITY AND CLINIC PARENTS
534. Maria Dung Cao: MR METABOLIC CHARACTERIZATION OF LOCALLY ADVANCED BREAST CANCER – TREATMENT EFFECTS AND PROGNOSIS
535. Mirta Mittelstedt Leal de Sousa: PROTEOMICS ANALYSIS OF PROTEINS INVOLVED IN DNA BASE REPAIR AND CANCER THERAPY
536. Halfdan Petursson: THE VALIDITY AND RELEVANCE OF INTERNATIONAL CARDIOVASCULAR DISEASE PREVENTION GUIDELINES FOR GENERAL PRACTICE
537. Marit By Rise: LIFTING THE VEIL FROM USER PARTICIPATION IN CLINICAL WORK – WHAT IS IT AND DOES IT WORK?

538. Lene Thoresen: NUTRITION CARE IN CANCER PATIENTS. NUTRITION ASSESSMENT: DIAGNOSTIC CRITERIA AND THE ASSOCIATION TO SURVIVAL AND HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH ADVANCED COLORECTAL CARCINOMA
539. Berit Doseth: PROCESSING OF GENOMIC URACIL IN MAN AND MOUSE
540. Gro Falkenér Bertheussen: PHYSICAL ACTIVITY AND HEALTH IN A GENERAL POPULATION AND IN CANCER SURVIVORS – METHODOLOGICAL, OBSERVATIONAL AND CLINICAL ASPECTS
541. Anne Kari Knudsen: CANCER PAIN CLASSIFICATION
542. Sjur Urdson Gjerald: A FAST ULTRASOUND SIMULATOR
543. Harald Edvard Mølmen Hansen: CARDIOVASCULAR EFFECTS OF HIGH INTENSITY AEROBIC INTERVAL TRAINING IN HYPERTENSITIVE PATIENTS, HEALTHY AGED AND YOUNG PERSONS
544. Sasha Gulati: SURGICAL RESECTION OF HIGH-GRADE GLIOMAS
545. John Chr. Fløvig: FREQUENCY AND EFFECT OF SUBSTANCES AND PSYCHOACTIVE MEDICATIONS THE WEEK BEFORE ADMISSION TO AN ACUTE PSYCHIATRIC DEPARTMENT
546. Kristin Moksnes Husby: OPTIMIZING OPIOID TREATMENT FOR CANCER PAIN – CLINICAL AND PHARMACOLOGICAL ASPECTS
547. Audun Hanssen-Bauer: X-RAY REPAIR CROSS-COMPLEMENTING PROTEIN 1 ASSOCIATED MULTIPROTEIN COMPLEXES IN BASE EXCISION REPAIR