Charles University in Prague

Faculty of Science

Department of Biochemistry

Ph.D. study program: Biochemistry

RNDr. Kamila Burdová

Molecular mechanisms underlying maintenance of genome stability

Summary of the thesis

Supervisor: RNDr. Pavel Janščák, CSc.

Laboratory of Genome Integrity

Institute of Molecular Genetics

Academy of Sciences of the Czech Republic

Prague, 2014

CONTENTS

1 ABSTRACT 3

2 INTRODUCTION 4

2.1 Mismatch Repair 4

2.2 Base Excision Repair 5

2.3 Double Strand Break Repair 5

2.3.1 Non-Homologous End-Joining 6

2.3.2 Homologous Recombination 7

2.3.3 Regulation of Double-Strand Break Repair 8

2.4 DNA Damage Response Signalling 8

2.5 RecQ Family of Helicases 9

3 AIMS 10

4 MATERIAL AND METHODS 11

5 RESULTS AND DISCUSSION 11

5.1 Involvement of Werner Syndrome Protein in MUTYH-Mediated Repair of Oxidative DNA Damage 11

5.2 Human RECQ5 Helicase Promotes Repair of DNA Double-Strand Breaks by Synthesis-Dependent Strand Annealing 12

5.3 DNA2 Cooperates with the WRN and BLM RecQ Helicases to Mediate Long-Range DNA-End Resection in Human Cells. 12

5.4 A role for the mismatch-binding factor MutSβ as a mediator of ATR activation in response to DNA double-strand breaks. 13

6 CONCLUSIONS 14

7 REFERENCES 14

8 CURRICULUM VITAE 18

1 ABSTRACT

Cells in our body are challenged every day by DNA damage arising as a result of both endogenous and exogenous insults. The ability of cells to repair DNA lesions is essential for correct propagation of genetic information. The most cytotoxic DNA lesion is DNA double-strand break (DSB) while oxidative DNA damage is one of the most frequent lesions. The aim of this thesis was to improve current the knowledge of the molecular mechanisms underlying the repair of DSBs and oxidative DNA damage.

The major source of oxidative damage in cells are reactive oxygen species that are constantly generated as by-products of cell metabolism. One of the most frequent lesions is 7,8-dihydro-8-oxo-guanine (8-oxo-G) that gives rise to 8-oxo-G:A mispairs during DNA replication and if left unrepaired, results in accumulation of DNA mutations. We found that WRN physically interacts with Polλ and stimulates DNA gap-filling by Polλ opposite to 8-oxo-G followed by strand displacement synthesis in MutY DNA glycosylase homolog (MUTYH) initiated base excision repair (BER) of 8-oxo-G:A mispairs.

There are two major pathways involved in repair of DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ). NHEJ is highly error-prone while HR is error-free. There are two subpathways of HR, namely synthesis-dependent strand annealing (SDSA) and canonical double strand break repair (DSBR). DSBR results in both crossovers (CO) and non-crossover (NCO) products while SDSA yields only NCOs. Undesired CO formation may lead to chromosomal rearrangements and loss of heterozygosity. Thus, in mitotic cells, majority of HR events proceed via SDSA to avoid crossing-over.

The molecular mechanism underlying promotion of SDSA is not well studied in human cells. RECQ5 and FBH1 have been suggested to be functional orthologs of Srs2 helicase that promotes SDSA in yeast cells. We have demonstrated that RECQ5 can prevent illegitimate RAD51 nucleofilament formation during post-synaptic phase of SDSA to promote formation of NCO products. Thus we propose that RECQ5 is the functional ortholog of Srs2 in human cells.

In yeast, Exo1 and Dna2 in conjunction with Sgs1 represent two separate pathways of long-range DNA end resection. In human cells, BLM helicase has been suggested to cooperate with DNA2 to mediate long-range resection. We were able to show that DNA2 cooperates with either BLM or WRN in human cells to mediate this process. In addition, our experiments suggest that BLM promotes DNA end resection in complex with TopIIIα-RMI1-RMI2.

Activation of ATR upon DSB induction depends on DNA end resection. We have identified the mismatch recognition complex MSH2-MSH3 as a component of the ATR signalling pathway. We have shown that MSH2-MSH3 is recruited to sites of DSBs in a DNA end resection dependent manner and promotes DSB repair by HR. Moreover, our results suggest that the MSH2-MSH3 complex binds to DNA hairpin structures in RPA-coated ssDNA and recruits the ATR-ATRIP complex hence stimulating ATR activation and DNA repair.

2 INTRODUCTION

Each cell in our body is everyday challenged by multiple deoxyribonucleic acid (DNA) damaging insults coming from both endogenous and exogenous sources. Endogenously, DNA damage comes from replication errors and oxygen radicals created by the cell metabolism. Exogenous sources include UV light, X-ray irradiation and genotoxic chemicals.

DNA lesions can be divided to subgroups depending on their nature. These involve damage of single bases or nucleotides, crosslinks and single and double strand breaks. There are different DNA repair mechanisms dealing with different types of DNA damage. These mechanisms are highly conserved from bacteria to mammals (Hoeijmakers, 2001).

Eukaryotic cells respond to DNA damage by multiple mechanisms that influence the cell in short term and long term. In short term, cells inhibit DNA metabolism such as transcription, replication and chromosome segregation. In longer perspective, there is barrier for cells not to divide with damaged DNA. This mechanism called cell cycle checkpoint is activated by DNA damage at different stages of the cell cycle and leads to transient or permanent cell cycle arrest (senescence). If the cell is not capable of repair and DNA lesion persists¨, it would undergo apoptosis and thus be eliminated from the organism. If all these mechanisms fail, then cells would divide even in presence of DNA damage leading to undesired mutations and chromosomal aberrations inherited by daughter cells. These can subsequently lead to cancer or accelerate the onset of aging (Hoeijmakers, 2001).

2.1 Mismatch Repair

One of the most important DNA repair systems in the cell is mismatch repair that deals mainly with replication errors such as adenine-guanine and thymidine-cytosine mismatches, and insertion – deletion loops (Jiricny, 2006). Mismatch repair is a multistep process involving mismatch recognition, excision, DNA resynthesis and ligation.

In the bacterium *Escherichia coli*, MutS homodimer binds to mismatch and recruits MutL homodimer that further recruits other factors needed to excise damaged DNA, fill-in the resulting DNA gap and ligate back to produce intact DNA molecule. In eukaryotes, five different MutS homologues (MSH) have been identified. Of these, MSH2, MSH3 and MSH6 were found to be present in heterodimeric complexes involved in the recognition of DNA lesions. Substrate specificities of these complexes are different but overlapping. MSH2-MSH3 complex (also named MutSβ) binds to loops of 1-14 extrahelical nucleotides while MSH2-MSH6 complex (also named MutSα) binds to base-base mismatches and single- and double-base insertions. Four different MutL homologues have been identified in humans: MLH1, MLH3, post-meiotic segregation protein-1 (PMS1) and PMS2 that form 3 different heterodimers: MLH1-MLH3 (MutLγ), MLH1-PMS1 (MutLβ) and MLH1-PMS2 (MutLα). Of these the MLH1-PMS2 heterodimer is of the most importance (Jiricny, 2006). The other factors needed for mismatch repair include homotrimeric

proliferating cell nuclear antigen (PCNA), DNA polymerase δ and/or DNA polymerase ε and exonuclease-I (EXO1).

Interestingly, the mismatch repair proteins MSH2, MSH3, MSH6 and MLH1 have been shown to be involved in the cellular response to DNA double strand breaks as they rapidly accumulate at sites of DNA damage caused by laser micro-irradiation (Hong et al., 2008; Liberti et al., 2010). As expected from canonical mismatch repair, MLH1 recruitment was found to be dependent on MSH2 (Jiricny, 2006). Experiments with *Msh2 -/-* mouse embryonic fibroblasts have shown potential role of MSH2 in homologous recombination (Bennardo et al., 2009). Moreover, MSH2 and MSH3 deficient cells were found to be more sensitive to DNA damage causing agents such as cis-platin, camptothecin and olaparib (Franchitto et al., 2003; Pichierri et al., 2001; Takahashi et al., 2011).

2.2 Base Excision Repair

Base excision repair (BER) is used when single base in DNA is damaged (e.g. alkylation, oxidation or deamination) and this base lesion is not distorting the DNA. More bulky base lesions that distort DNA helix are repaired by different mechanism called nucleotide excision repair (NER). One of the most common lesions is 7,8-dihydro-8-oxo-guanine (8-oxo-G) with steady state level of about 10^3 lesions per cell per day in normal tissue (Klungland et al., 1999). If unrepaired, replication of DNA with these lesions frequently lead to 8-oxo-G:A mispair formation and subsequent G:C and T:A transversion mutations (Avkin and Livneh, 2002).

In human cells, 8-oxo-G:A mispairs are repaired by two subsequent BER events (van Loon et al., 2010). In the first step, excision of mispaired A base by MutY glycosylase homolog (MUTYH) in reaction coordinated with PCNA occurs and is followed by cleavage of AP site by APE1 (Hayashi et al., 2002; Takao et al., 1999; van Loon et al., 2010; Yang et al., 2001). Next, DNA polymerase λ (Polλ) incorporates dCTP opposite to 8-oxo-G site in the presence of PCNA and RPA with subsequent strand-displacement synthesis. The resulting 5’ ssDNA strand flap is cleaved by FEN1 that is recruited to the site of damage by. Finally, the nick in DNA generated by FEN1 cleavage is sealed by Ligase I (Maga et al., 2008; Maga et al., 2007; van Loon et al., 2010). 8-oxo-G:C mispair is predominantly recognized and 8-oxo-G is excised by OGG1 glycosylase during short patch BER event (Hazra et al., 2007).

2.3 Double Strand Break Repair

There are two major pathways involved in double strand break (DSB) repair: non-homologous end joining (NHEJ) and homologous recombination (HR) (Figure 1). Binding of MRE11- RAD50-NBS1/Xrs2 (MRN/X) complex to ends of DSB is the first step of DSB repair and is common to all pathways (Lisby et al., 2004). Following steps differ in each pathway and usually inhibit each other.

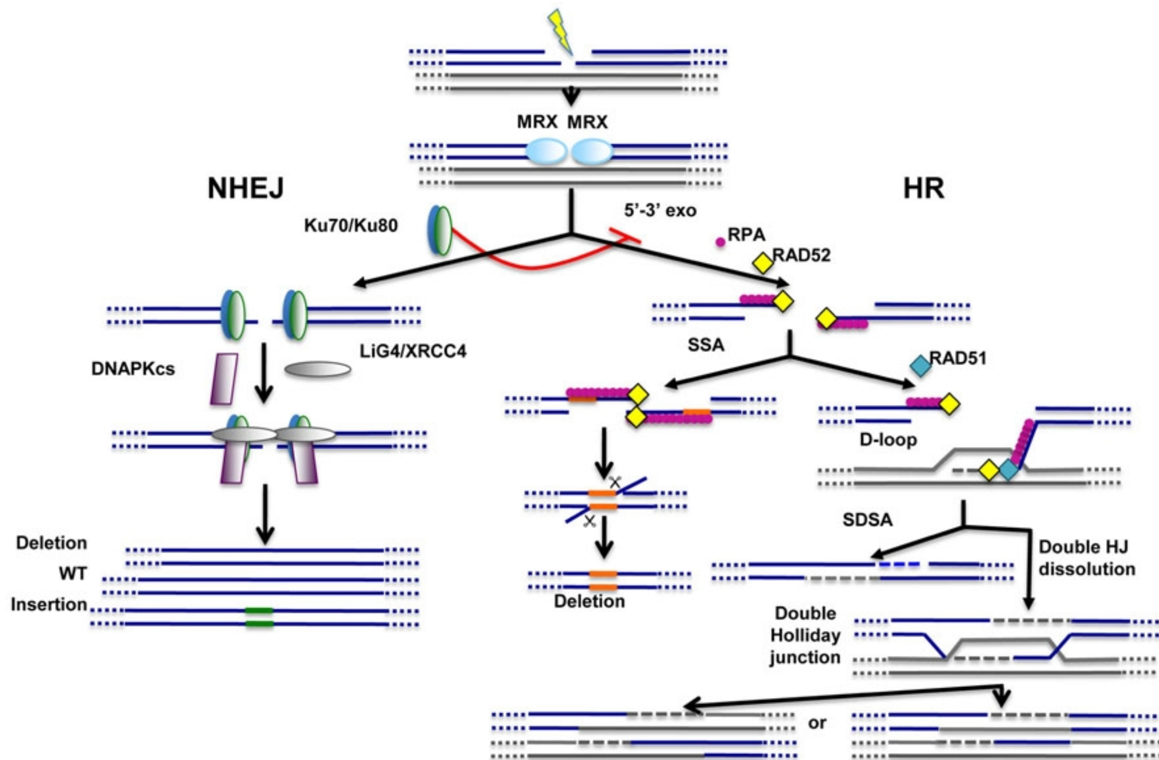

Figure 1. Major double-strand break repair pathways: Non-homologous end-joining (NHEJ) and homologous recombination (HR). MRE11/RAD50/XRS2 (MRX) complex is recruited at DNA ends. Ku70/Ku80 heterodimer is required for NHEJ and by inhibition of DNA end resection represses HR. 5'-3' resection is the first step of HR producing ssDNA that is coated by RPA. Single strand annealing (SSA) occurs only if direct repeats are present around the site of DSB resulting in loss of DNA. Further steps of HR involve exchange of RPA for RAD51, homology search and strand invasion. Two subpathways of HR represent synthesis-dependent strand annealing (SDSA) and canonical double-strand break repair. Adapted from (Decottignies, 2013).

2.3.1 Non-Homologous End-Joining

In general, non-homologous end joining (NHEJ) involves direct ligation of free DNA ends or small alterations of DNA ends (excision and/or synthesis) prior to ligation if they cannot be directly re-ligated (Lieber, 2010; van Gent and van der Burg, 2007). Firstly KU70-KU80 heterodimer binds to DNA ends preventing their degradation and recruits DNA-protein kinase catalytic subunit (DNA-PKcs) forming DNA-PK holoenzyme and other end processing enzymes, such as Artemis, polynucleotide kinase, and terminal deoxynucleotidyl transferase (Lieber, 2010). The ligation step is performed by Ligase IV in complex with X-ray repair cross-complementing protein 4 (XRCC4) and XRCC4-like factor (XLF) complex (Lieber, 2010; van Gent and van der Burg, 2007).

Over last years, new pathway called alternative end-joining (alt-EJ) and microhomology-mediated end joining (MMEJ) were discovered and proven to play role in double-strand break repair in vivo (Decottignies, 2013; Mladenov and Iliakis, 2011). Both these pathways are

repressed by KU70-KU80 heterodimer and involve 5' to 3' DNA end resection catalysed by MRN complex with CtIP and/or EXO1.

2.3.2 Homologous Recombination

Homologous recombination (HR) is most commonly used during replication promoting replication fork restart or one-ended DSB repair. However it is also used for repair of two-ended double strand breaks (Petermann and Helleday, 2010). HR begins with 5' to 3' DNA end resection mediated by MRN complex in conjunction with CtIP (Longhese et al., 2010; Sartori et al., 2007). The length of resected DNA is up to 100bp (Symington and Gautier, 2011). The second step of resection (long range resection) that is in mediated by EXO1 exonuclease or by joint action of DNA2 endonuclease and a RecQ family helicase, Sgs1 in budding yeast (Cejka et al., 2010a; Mimitou and Symington, 2008; Nimonkar et al., 2011; Niu et al., 2010; Zhu et al., 2008). In human cells it has been suggested that Bloom helicase (BLM) is the Sgs1 ortholog mediating long range resection (Nimonkar et al., 2011). However in *Xenopus laevis* model organism, it has been shown that xWRN helicase interacts with DNA2 and promotes resection of DNA ends *in vitro* (Liao et al., 2008; Toczylowski and Yan, 2006; Yan et al., 2005). Resected DNA yields long stretches of single stranded DNA (ssDNA) that are bound by trimeric replication protein A (RPA) to prevent nucleolytic degradation (San Filippo et al., 2008). RPA is exchanged with help of BRCA2 for RAD51 recombinase forming RAD51-nucleofilament that conducts the search for homologous DNA sequence (Thorslund et al., 2010). After the invasion of RAD51-nucleofilament to the donor sister chromatid a joined DNA molecule is formed, termed displacement (D) -loop that can be extended by DNA strand and repair synthesis (San Filippo et al., 2008).

This structure can be resolved by two distinct mechanisms, namely synthesis dependent strand annealing (SDSA) and canonical double strand break repair (DSBR) (Heyer et al., 2010; San Filippo et al., 2008). During SDSA the extended D-loop is disrupted by action of helicase, newly synthesized DNA strand is released and annealed back to the other end of damaged DNA (Kass and Jasin, 2010; Nassif et al., 1994; Paques and Haber, 1999; Uringa et al., 2011). Gaps are then filed by gap-filling DNA synthesis and DNA ends are ligated together (Nassif et al., 1994). SDSA yields only non-crossover products (Heyer et al., 2010).

The canonical DSBR starts if the second DSB end is captured by the D-loop to form an intermediate with two Holliday junctions, referred to as double Holliday junction (dHJ). This structure can be either resolved by specialized endonucleases including human GEN1, MUS81/EME1, SLX1/SLX4 or dissolved by concerted actions of the BLM helicase and the DNA topoisomerase IIIα (TOPOIIIα) in complex with RMI1 and RMI2 (Cejka et al., 2010b; Wechsler et al., 2011). While resolution of dHJ can result both in crossovers (COs) and non-crossovers (NCOs), dissolution yields only non-crossovers (Heyer et al., 2010).

Apart from HR, there is a more specific repair pathway called single strand annealing (SSA) that is used when direct repeats are present around the site of DSB. SSA involves long range resection of DNA ends that are subsequently coated by RPA (Cannavo et al., 2013). Annealing of

complementary sequences with more than 30 nucleotides is mediated by RAD52, non-complementary 3' overhangs are cleaved by ERCC1-XPF complex in conjunction with SLX4 and DNA is ligated resulting in one repeat deletion (Lyndaker and Alani, 2009; Sugawara et al., 2000). This process is RAD51 independent as no template DNA is used for repair.

2.3.3 Regulation of Double-Strand Break Repair

While NHEJ, SSA and MMEJ are error-prone mechanisms leading to insertions and/or deletions around the site of DSB, HR is mostly error-free (Karanam et al., 2012). Repair pathway choice is largely dependent on cell cycle phase and chromatin compaction at the site of DSB (Goodarzi et al., 2010; Kass and Jasin, 2010). NHEJ and MMEJ repair pathways are active throughout the cell cycle while HR is restricted to S phase and G2 phase cells due to the need of homologous sequence as a repair template that is preferably provided by sister chromatid (Heyer et al., 2010; Kass and Jasin, 2010). This is ensured by regulation of protein expression during cell cycle and by protein phosphorylations mediated by cyclin-dependent kinases (CDKs) that positively and negatively regulate HR (Heyer et al., 2010; Kass and Jasin, 2010).

The other important protein that regulate DSB repair is 53BP1 with its recently discovered binding partner Rif1 that binds to modified histones present on chromatin around double strand break where it promotes NHEJ in G1 cells and blocks DNA end resection thus inhibiting HR (Zimmermann and de Lange, 2014; Zimmermann et al., 2013).

2.4 DNA Damage Response Signalling

Depending on type of DNA damage, different members of the family of phosphatidylinositol 3-like kinases are activated and their activity is usually enhanced by autophosphorylations. There are three members of this family: Ataxia telangiectasia mutated (ATM), Ataxia telangiectasia and Rad3 related (ATR) and DNA-PKcs. ATM kinase is of the most importance after DNA damage, however, ATR kinase can be also activated under certain circumstances.

During DSB repair, ATM is recruited to site of damage by MRN complex that is bound to blunt ended or minimally processed DSB ends. ATM then phosphorylates checkpoint kinase protein 2 (CHK2) and p53 leading to cell cycle arrest, activation of proteins involved in DNA damage repair and, in case of a high load of DNA damage, to apoptosis (Goodarzi and Jeggo, 2013). Other important substrate of ATM is histone isoform H2AX that is phosphorylated at serine 139 (γ-H2AX). γ-H2AX signal spreads even a megabase away from DSB end and serves as a marker of DNA damage in cells (Goodarzi and Jeggo, 2013). During HR, resection of DNA leads to generation of ssDNA coated by RPA that recruits ATR kinase in complex with ATRIP (Cimprich and Cortez, 2008). Activity of ATR kinase is stimulated by its interaction with DNA topoisomerase 2-binding protein 1 (TopBP1) and RAD9-HUS1-RAD1 (9-1-1) complex that are bound to ssDNA-

dsDNA junction and phosphorylates checkpoint kinase 1 (CHK1) that leads to cell cycle arrest through inhibition of CDC25 (Cimprich and Cortez, 2008).

2.5 RecQ Family of Helicases

Helicases are enzymes that catalyze unwinding of nucleic acid duplexes using energy derived from hydrolysis of nucleoside triphosphates (Singleton et al., 2007). There are two classes of helicases: type A that translocates in 3‘ to 5‘ direction and type B that translocates in opposite direction. Helicases have been also divided to six superfamilies (SF1-6) based on amino acid sequence homology (Singleton et al., 2007). RecQ helicases are highly conserved from bacteria to humans. They belong to the SF2 superfamily of helicases and are according to the *Escherichia coli* RecQ helicase. Most unicelullar organism have only one RecQ homolog (e.g. Sgs1 in *Saccharomyces cerevisiae*, while multicellular organisms express two and more RecQ homologues (Bernstein et al., 2010). In humans there are five members of RecQ family of helicases, namely RECQL1, WRN, BLM, RECQL4 and RECQL5β (Figure 2). RecQ helicases play role in replication, transcription, telomere maintenance and DNA damage repair and thus are important in maintenance of genome stability.

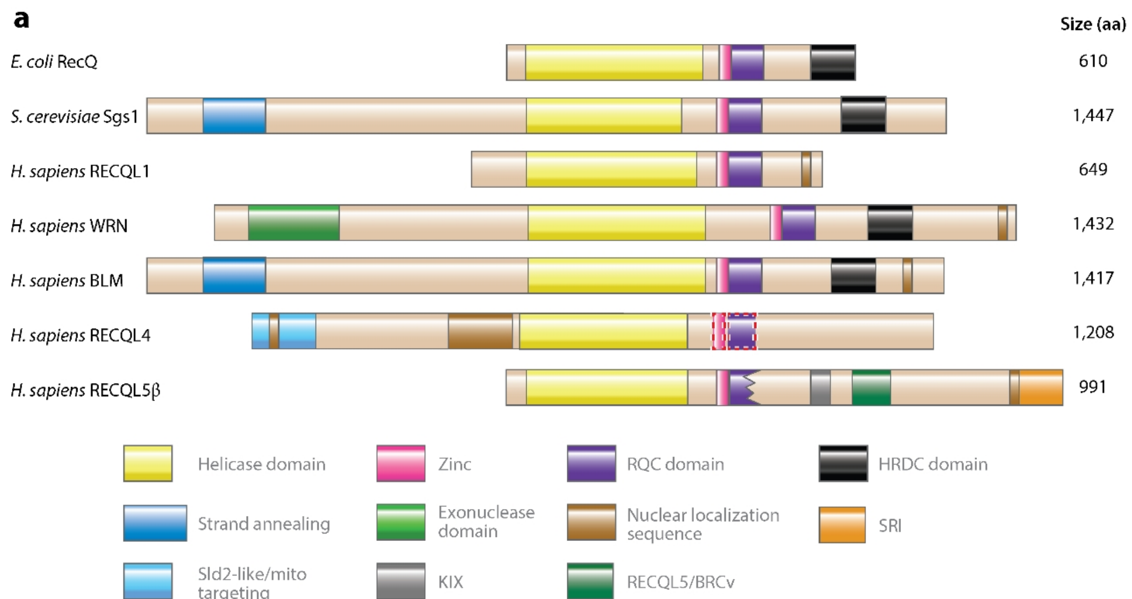

Figure 2. The domain structure of RecQ helicases from *E.coli, S.cerevisiae* and *H.sapiens*. Adapted from (Croteau et al., 2014).

Mutations in three human RecQ helicases, namely WRN, BLM and RECQL4 have been found in hereditary genetic diseases that are characterized by cancer predisposition and/or premature aging (Bernstein et al., 2010). Werner syndrome (WS) is found in patients with mutation in *WRN* helicase and is characterized by early onset of age-associated pathologies such as cataracts and

osteoporosis. Symptoms of WS start to appear in early 20s and result in death around 46-54 years. Tumours of WS patients are mainly of mesenchymal origin (e.g. sarcomas). Cells derived from WS patients show marks of genomic instability such as increased frequency of chromosomal rearrangements (Bernstein et al., 2010).

BLM helicase is mutated in Bloom syndrome (BS) that is characterized by dwarfism due to pre- and post-natal growth retardation. Beside this feature, patients with BS are light sensitive, show immunodeficiency, male infertility and predisposition to all types of cancer. Cells derived from BS patients show increased level of spontaneous chromatid breaks/gaps, elevated rates of mutations, and most typically increased frequency of exchanges between homologous chromosomes and sister chromatids (SCEs) (Bernstein et al., 2010). Mutations in *RECQL4* have been associated with Rothmund-Thompson syndrome type II, RAPADILINO syndrome and Baller-Gerold syndrome (Bernstein et al., 2010).

3 AIMS

1. Cells derived from Werner syndrome patients accumulate more 8-oxo-G lesions compared to cells with normal WRN expression. Molecular basis of this process is not known. **Therefore the first aim was to elucidate the role of WRN helicase in oxidative damage repair.**

2. Preferential use of SDSA sub-pathway during HR in interphase cells is important for maintenance of genomic stability as it leads to non-crossover products only. In yeast, there are two helicases that were found responsible for SDSA promotion, however their functional orthologs in mammalian cells were not described. Question to be addressed was: **Which helicase is responsible for SDSA sub-pathway promotion in human cells?**

3. Process of 5' to 3' DNA end resection during HR has been extensively studied in yeast but is not fully understood in mammalian cells. **Therefore, the goal of this part of the thesis was to explore this process in human cells.**

4. Mismatch repair recognition complexes as well as other proteins were found to be recruited to DSBs created by laser micro-irradiation. There is supportive evidence that they are somehow involved in double-strand break repair as deficient cells are more sensitive to agents causing DSBs. **The aim was to clarify the function of MSH2 complexes in the cellular response to DSBs.**

4 MATERIAL AND METHODS

- Cloning and standard molecular biology techniques
- Recombinant protein expression and purification
- Tissue cultures, transfections, preparation of stable cell lines
- Cell based reporter assays
- Cell fractionation
- Kinase assays
- Immunocytochemistry and fluorescence microscopy
- Flow cytometry
- SDS-PAGE and immunoblotting
- Co-immunoprecipitations and pull-downs
- RNA isolation, cDNA synthesis and quantitative RT-PCR

5 RESULTS AND DISCUSSION

5.1 INVOLVEMENT OF WERNER SYNDROME PROTEIN IN MUTYH-MEDIATED REPAIR OF OXIDATIVE DNA DAMAGE

Involvement of Werner syndrome protein in MUTYH-mediated repair of oxidative DNA damage.

Kanagaraj R, Parasuraman P, Mihaljevic B, van Loon B, Burdova K, König C, Furrer A, Bohr VA, Hübscher U, Janscak P.

Nucleic Acids Res. 2012 Sep 1; 40(17):8449-59.

IF: 8.808

Abstract:

Reactive oxygen species constantly generated as by-products of cellular metabolism readily attack genomic DNA creating mutagenic lesions such as 7,8-dihydro-8-oxo-guanine (8-oxo-G) that promote aging. 8-oxo-G:A mispairs arising during DNA replication are eliminated by base excision repair initiated by the MutY DNA glycosylase homologue (MUTYH). Here, by using formaldehyde crosslinking in mammalian cell extracts, we demonstrate that the WRN helicase/exonuclease defective in the premature aging disorder Werner syndrome (WS) is recruited to DNA duplex containing an 8-oxo-G:A mispair in a manner dependent on DNA polymerase λ (Polλ) that catalyzes accurate DNA synthesis over 8-oxo-G. Similarly, by immunofluorescence, we show that Polλ is required for accumulation of WRN at sites of 8-oxo-G lesions in human cells. Moreover, we show that nuclear focus formation of WRN and Polλ

induced by oxidative stress is dependent on ongoing DNA replication and on the presence of MUTYH. Cell viability assays reveal that depletion of MUTYH suppresses the hypersensitivity of cells lacking WRN and/or Polλ to oxidative stress. Biochemical studies demonstrate that WRN binds to the catalytic domain of Polλ and specifically stimulates DNA gap filling by Polλ over 8-oxo-G followed by strand displacement synthesis. Our results suggest that WRN promotes long-patch DNA repair synthesis by Polλ during MUTYH-initiated repair of 8-oxo-G:A mispairs.

5.2 Human RECQ5 Helicase Promotes Repair of DNA Double-Strand Breaks by Synthesis-Dependent Strand Annealing

Human RECQ5 helicase promotes repair of DNA double-strand breaks by synthesis-dependent strand annealing.

Paliwal S, Kanagaraj R, Sturzenegger A, Burdova K, Janscak P.

Nucleic Acids Res. 2014 Feb; 42(4):2380-90. doi: 10.1093/nar/gkt1263.

IF: 8.808

Abstract:

Most mitotic homologous recombination (HR) events proceed via a synthesis-dependent strand annealing mechanism to avoid crossing over, which may give rise to chromosomal rearrangements and loss of heterozygosity. The molecular mechanisms controlling HR sub-pathway choice are poorly understood. Here, we show that human RECQ5, a DNA helicase that can disrupt RAD51 nucleoprotein filaments, promotes formation of noncrossover products during DNA double-strand break-induced HR and counteracts the inhibitory effect of RAD51 on RAD52-mediated DNA annealing *in vitro* and *in vivo*. Moreover, we demonstrate that RECQ5 deficiency is associated with an increased occupancy of RAD51 at a double-strand break site, and it also causes an elevation of sister chromatid exchanges on inactivation of the Holliday junction dissolution pathway or on induction of a high load of DNA damage in the cell. Collectively, our findings suggest that RECQ5 acts during the post-synaptic phase of synthesis-dependent strand annealing to prevent formation of aberrant RAD51 filaments on the extended invading strand, thus limiting its channeling into potentially hazardous crossover pathway of HR.

5.3 DNA2 Cooperates with the WRN and BLM RecQ Helicases to Mediate Long-Range DNA-End Resection in Human Cells.

DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA-end resection in human cells.

Sturzenegger A*, Burdova K*, Kanagaraj R, Levikova M, Pinto C, Cejka P, Janscak P.

J Biol Chem. 2014 Sep 26;289(39):27314-26. doi: 10.1074/jbc.M114.578823.

IF: 4.651

Abstract:

The 5’-3’ resection of DNA ends is a prerequisite for the repair of DNAdouble strand breaks by homologous recombination, microhomology-mediated end joining, and single strand annealing. Recent studies in yeast have shown that, following initial DNA end processing by the Mre11-Rad50-Xrs2 complex and Sae2, the extension of resection tracts is mediated either by exonuclease 1 or by combined activities of the RecQ family DNA helicase Sgs1 and the helicase/endonuclease Dna2. Although human DNA2 has been shown to cooperate with the BLM helicase to catalyze the resection of DNA ends, it remains a matter of debate whether another human RecQ helicase, WRN, can substitute for BLM in DNA2-catalyzed resection. Here we present evidence that WRN and BLM act epistatically with DNA2 to promote the long-range resection of double strand break ends in human cells. Our biochemical experiments show that WRN and DNA2 interact physically and coordinate their enzymatic activities to mediate 5’-3’ DNA end resection in a reaction dependent on RPA. In addition, we present in vitro and in vivo data suggesting that BLM promotes DNA end resection as part of the BLM-TOPOIIIα-RMI1-RMI2 complex. Our study provides new mechanistic insights into the process of DNA end resection in mammalian cells.

5.4 A role for the mismatch-binding factor MutSβ as a mediator of ATR activation in response to DNA double-strand breaks.

A role for the mismatch-binding factor MutSβ as a mediator of ATR activation in response to DNA double-strand breaks.

Burdova K*, Mihaljevic B*, Janscak P

Mol Cell, submitted

IF: 14.464

Abstract:

Ataxia telangiectasia and Rad3-related (ATR) protein kinase, a master regulator of DNA damage response, is activated by RPA-coated single-stranded DNA (ssDNA) generated at stalled replication forks or DNA double-strand breaks (DSBs). Here we identify the mismatch-binding protein MutSβ, a heterodimer of MSH2 and MSH3, as a key component of the ATR signaling cascade. MutSβ accumulates at sites of DSBs in a manner dependent on DNA-end resection and promotes DSB repair by homologous recombination. MSH2 and MSH3 form a complex with ATR and its regulatory partner ATRIP, and their depletion compromises the formation of ATRIP foci and the phosphorylation of ATR substrates in cells responding to replication-associated DSBs. Purified MutSβ heterodimer binds to DNA secondary structures persisting in RPA-ssDNA complexes and promotes ATRIP recruitment. These results suggest that MutSβ mediates binding

of the ATR-ATRIP complex to RPA-coated ssDNA at sites of DNA damage to promote ATR activation and DNA repair.

6 CONCLUSIONS

WRN STIMULATES REPAIR OF 8-OXO-G:A MISPAIRS

Polλ recruits WRN to 8-oxo-G:A mispairs in vitro. WRN localizes to sites of oxidative damage in S phase cells and interacts with Polλ. Co-depletion of MUTYH glycosylase rescues sensitivity of WRN and Polλ depleted cells to hydrogen peroxide. WRN stimulates DNA repair synthesis activity of Polλ.

RECQ5 PROMOTES SDSA SUBPATWAY OF HR IN HUMAN CELLS

RECQ5 promotes non-crossover product formation during HR in human cells. RECQ5 helicase counteracts the inhibitory effect of RAD51 on RAD52-mediated ssDNA annealing in vitro. Depletion of RECQ5 leads to accumulation of RAD51 at chromatin flanking the sites of DSBs.

WRN INTERACTS WITH DNA2 DURING DNA END RESECTION AT DSBS

WRN-DNA2 resects DNA ends more efficiently than BLM-DNA2 in vitro. The helicase function of WRN and endonuclease activity are necessary for DNA end resection. WRN and DNA2 form complex in vivo and in vitro. Two long-range resection pathways exist in HEK293 cells: EXO1 and WRN-DNA2. BLM acts mainly as an antirecombinase in HEK293 cells. In U2OS cells, DNA2 cooperates with WRN or BLM in long-range resection. TopIIIα-RMI1-RMI2 complex stimulates BLM-DNA2 mediated DNA end resection in vitro and in vivo.

MSH2-MSH3 COMPLEX PROMOTES ATR ACTIVATION AND REPAIR OF DNA DOUBLE-STRAND BREAKS

MSH2-MSH3 complex is involved in DSB repair by HR. MSH2-MSH3 interacts with ATR-ATRIP complex. MSH2-MSH3 stimulates ATR activation and promotes ATRIP foci formation after DNA damage. MSH2-MSH3 binds to DNA secondary structures in RPA-ssDNA complexes and promotes ATRIP recruitment.

7 REFERENCES

Avkin, S., and Livneh, Z. (2002). Efficiency, specificity and DNA polymerase-dependence of translesion replication across the oxidative DNA lesion 8-oxoguanine in human cells. Mutation research *510*, 81-90.

Bennardo, N., Gunn, A., Cheng, A., Hasty, P., and Stark, J.M. (2009). Limiting the persistence of a chromosome break diminishes its mutagenic potential. PLoS genetics *5*, e1000683.

Bernstein, K.A., Gangloff, S., and Rothstein, R. (2010). The RecQ DNA helicases in DNA repair. Annual review of genetics *44*, 393-417.

Cannavo, E., Cejka, P., and Kowalczykowski, S.C. (2013). Relationship of DNA degradation by Saccharomyces cerevisiae exonuclease 1 and its stimulation by RPA and Mre11-Rad50-Xrs2 to DNA end resection. Proc Natl Acad Sci U S A *110*, E1661-1668.
Cejka, P., Cannavo, E., Polaczek, P., Masuda-Sasa, T., Pokharel, S., Campbell, J.L., and Kowalczykowski, S.C. (2010a). DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. Nature *467*, 112-116.
Cejka, P., Plank, J.L., Bachrati, C.Z., Hickson, I.D., and Kowalczykowski, S.C. (2010b). Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. Nat Struct Mol Biol *17*, 1377-1382.
Cimprich, K.A., and Cortez, D. (2008). ATR: an essential regulator of genome integrity. Nature reviews. Molecular cell biology *9*, 616-627.
Croteau, D.L., Popuri, V., Opresko, P.L., and Bohr, V.A. (2014). Human RecQ helicases in DNA repair, recombination, and replication. Annual review of biochemistry *83*, 519-552.
Decottignies, A. (2013). Alternative end-joining mechanisms: a historical perspective. Frontiers in genetics *4*, 48.
Franchitto, A., Pichierri, P., Piergentili, R., Crescenzi, M., Bignami, M., and Palitti, F. (2003). The mammalian mismatch repair protein MSH2 is required for correct MRE11 and RAD51 relocalization and for efficient cell cycle arrest induced by ionizing radiation in G2 phase. Oncogene *22*, 2110-2120.
Goodarzi, A.A., Jeggo, P., and Lobrich, M. (2010). The influence of heterochromatin on DNA double strand break repair: Getting the strong, silent type to relax. DNA repair *9*, 1273-1282.
Goodarzi, A.A., and Jeggo, P.A. (2013). The repair and signaling responses to DNA double-strand breaks. Advances in genetics *82*, 1-45.
Hayashi, H., Tominaga, Y., Hirano, S., McKenna, A.E., Nakabeppu, Y., and Matsumoto, Y. (2002). Replication-associated repair of adenine:8-oxoguanine mispairs by MYH. Current biology : CB *12*, 335-339.
Hazra, T.K., Das, A., Das, S., Choudhury, S., Kow, Y.W., and Roy, R. (2007). Oxidative DNA damage repair in mammalian cells: a new perspective. DNA repair *6*, 470-480.
Heyer, W.D., Ehmsen, K.T., and Liu, J. (2010). Regulation of homologous recombination in eukaryotes. Annual review of genetics *44*, 113-139.
Hoeijmakers, J.H. (2001). Genome maintenance mechanisms for preventing cancer. Nature *411*, 366-374.
Hong, Z., Jiang, J., Hashiguchi, K., Hoshi, M., Lan, L., and Yasui, A. (2008). Recruitment of mismatch repair proteins to the site of DNA damage in human cells. J Cell Sci *121*, 3146-3154.
Jiricny, J. (2006). The multifaceted mismatch-repair system. Nature reviews. Molecular cell biology *7*, 335-346.
Karanam, K., Kafri, R., Loewer, A., and Lahav, G. (2012). Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Mol Cell *47*, 320-329.
Kass, E.M., and Jasin, M. (2010). Collaboration and competition between DNA double-strand break repair pathways. FEBS letters *584*, 3703-3708.
Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T., and Barnes, D.E. (1999). Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci U S A *96*, 13300-13305.

Liao, S., Toczylowski, T., and Yan, H. (2008). Identification of the Xenopus DNA2 protein as a major nuclease for the 5'->3' strand-specific processing of DNA ends. Nucleic acids research *36*, 6091-6100.
Liberti, S.E., Andersen, S.D., Wang, J., May, A., Miron, S., Perderiset, M., Keijzers, G., Nielsen, F.C., Charbonnier, J.B., Bohr, V.A.*, et al.* (2010). Bi-directional routing of DNA mismatch repair protein human exonuclease 1 to replication foci and DNA double strand breaks. DNA repair *10*, 73-86.
Lieber, M.R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annual review of biochemistry *79*, 181-211.
Lisby, M., Barlow, J.H., Burgess, R.C., and Rothstein, R. (2004). Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell *118*, 699-713.
Longhese, M.P., Bonetti, D., Manfrini, N., and Clerici, M. (2010). Mechanisms and regulation of DNA end resection. The EMBO journal *29*, 2864-2874.
Lyndaker, A.M., and Alani, E. (2009). A tale of tails: insights into the coordination of 3' end processing during homologous recombination. Bioessays *31*, 315-321.
Maga, G., Crespan, E., Wimmer, U., van Loon, B., Amoroso, A., Mondello, C., Belgiovine, C., Ferrari, E., Locatelli, G., Villani, G.*, et al.* (2008). Replication protein A and proliferating cell nuclear antigen coordinate DNA polymerase selection in 8-oxo-guanine repair. Proc Natl Acad Sci U S A *105*, 20689-20694.
Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E., Bertocci, B., and Hubscher, U. (2007). 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Nature *447*, 606-608.
Mimitou, E.P., and Symington, L.S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature *455*, 770-774.
Mladenov, E., and Iliakis, G. (2011). Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutation research *711*, 61-72.
Nassif, N., Penney, J., Pal, S., Engels, W.R., and Gloor, G.B. (1994). Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. Molecular and cellular biology *14*, 1613-1625.
Nimonkar, A.V., Genschel, J., Kinoshita, E., Polaczek, P., Campbell, J.L., Wyman, C., Modrich, P., and Kowalczykowski, S.C. (2011). BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. Genes Dev *25*, 350-362.
Niu, H., Chung, W.H., Zhu, Z., Kwon, Y., Zhao, W., Chi, P., Prakash, R., Seong, C., Liu, D., Lu, L.*, et al.* (2010). Mechanism of the ATP-dependent DNA end-resection machinery from Saccharomyces cerevisiae. Nature *467*, 108-111.
Paques, F., and Haber, J.E. (1999). Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiology and molecular biology reviews : MMBR *63*, 349-404.
Petermann, E., and Helleday, T. (2010). Pathways of mammalian replication fork restart. Nature reviews. Molecular cell biology *11*, 683-687.
Pichierri, P., Franchitto, A., Piergentili, R., Colussi, C., and Palitti, F. (2001). Hypersensitivity to camptothecin in MSH2 deficient cells is correlated with a role for MSH2 protein in recombinational repair. Carcinogenesis *22*, 1781-1787.
San Filippo, J., Sung, P., and Klein, H. (2008). Mechanism of eukaryotic homologous recombination. Annual review of biochemistry *77*, 229-257.

Sartori, A.A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., and Jackson, S.P. (2007). Human CtIP promotes DNA end resection. Nature *450*, 509-514.
Singleton, M.R., Dillingham, M.S., and Wigley, D.B. (2007). Structure and mechanism of helicases and nucleic acid translocases. Annual review of biochemistry *76*, 23-50.
Sugawara, N., Ira, G., and Haber, J.E. (2000). DNA length dependence of the single-strand annealing pathway and the role of Saccharomyces cerevisiae RAD59 in double-strand break repair. Molecular and cellular biology *20*, 5300-5309.
Symington, L.S., and Gautier, J. (2011). Double-strand break end resection and repair pathway choice. Annual review of genetics *45*, 247-271.
Takahashi, M., Koi, M., Balaguer, F., Boland, C.R., and Goel, A. (2011). MSH3 mediates sensitization of colorectal cancer cells to cisplatin, oxaliplatin, and a poly(ADP-ribose) polymerase inhibitor. The Journal of biological chemistry *286*, 12157-12165.
Takao, M., Zhang, Q.M., Yonei, S., and Yasui, A. (1999). Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase. Nucleic acids research *27*, 3638-3644.
Thorslund, T., McIlwraith, M.J., Compton, S.A., Lekomtsev, S., Petronczki, M., Griffith, J.D., and West, S.C. (2010). The breast cancer tumor suppressor BRCA2 promotes the specific targeting of RAD51 to single-stranded DNA. Nat Struct Mol Biol *17*, 1263-1265.
Toczylowski, T., and Yan, H. (2006). Mechanistic analysis of a DNA end processing pathway mediated by the Xenopus Werner syndrome protein. The Journal of biological chemistry *281*, 33198-33205.
Uringa, E.J., Youds, J.L., Lisaingo, K., Lansdorp, P.M., and Boulton, S.J. (2011). RTEL1: an essential helicase for telomere maintenance and the regulation of homologous recombination. Nucleic acids research *39*, 1647-1655.
van Gent, D.C., and van der Burg, M. (2007). Non-homologous end-joining, a sticky affair. Oncogene *26*, 7731-7740.
van Loon, B., Markkanen, E., and Hubscher, U. (2010). Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. DNA repair *9*, 604-616.
Wechsler, T., Newman, S., and West, S.C. (2011). Aberrant chromosome morphology in human cells defective for Holliday junction resolution. Nature *471*, 642-646.
Yan, H., McCane, J., Toczylowski, T., and Chen, C. (2005). Analysis of the Xenopus Werner syndrome protein in DNA double-strand break repair. J Cell Biol *171*, 217-227.
Yang, H., Clendenin, W.M., Wong, D., Demple, B., Slupska, M.M., Chiang, J.H., and Miller, J.H. (2001). Enhanced activity of adenine-DNA glycosylase (Myh) by apurinic/apyrimidinic endonuclease (Ape1) in mammalian base excision repair of an A/GO mismatch. Nucleic acids research *29*, 743-752.
Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. (2008). Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell *134*, 981-994.
Zimmermann, M., and de Lange, T. (2014). 53BP1: pro choice in DNA repair. Trends in cell biology *24*, 108-117.
Zimmermann, M., Lottersberger, F., Buonomo, S.B., Sfeir, A., and de Lange, T. (2013). 53BP1 regulates DSB repair using Rif1 to control 5' end resection. Science *339*, 700-704.

8 CURRICULUM VITAE

Personal data:

Kamila Burdová
born on 3rd February 1984 in Prague, Czech Republic

Current address:

Dept. of Genome Integrity, Institute of Molecular Genetics, AS CR, v.v.i.
Videnska 1083, 142 20 Praha 4
burdova@img.cas.cz
tel.: +420 241 063 112

Education:

2008 – present: Dept. of Genome Integrity, Institute of Molecular Genetics, AS CR, v.v.i., PhD student, supervisor: RNDr. Pavel Janščák, CSc.

2006 – 2008: Charles University in Prague, Faculty of Science, Department of Biochemistry, master degree (Diploma thesis: Selected chemopreventive compounds as cytochrome P450 inducers, supervisor: Prof. RNDr. Petr Hodek, CSc.)

2003 – 2006 Charles University in Prague, Faculty of Science, Department of Biochemistry, bachelor degree (Bachelor thesis: The influence of chemopreventive compounds on food carcinogen activation, supervisor: Prof. RNDr. Petr Hodek, CSc.)

Publications included in the Ph.D. thesis:

Involvement of Werner syndrome protein in MUTYH-mediated repair of oxidative DNA damage.
Kanagaraj R, Parasuraman P, Mihaljevic B, van Loon B, Burdova K, König C, Furrer A, Bohr VA, Hübscher U, Janscak P.
Nucleic Acids Res. 2012 Sep 1; 40(17):8449-59.

Human RECQ5 helicase promotes repair of DNA double-strand breaks by synthesis-dependent strand annealing.
Paliwal S, Kanagaraj R, Sturzenegger A, Burdova K, Janscak P.
Nucleic Acids Res. 2014 Feb; 42(4):2380-90. doi: 10.1093/nar/gkt1263.

DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA-end resection in human cells.
Sturzenegger A*, Burdova K*, Kanagaraj R, Levikova M, Pinto C, Cejka P, Janscak P.
J Biol Chem. 2014 Sep 26;289(39):27314-26. doi: 10.1074/jbc.M114.578823.

A role for the mismatch-binding factor MutSβ as a mediator of ATR activation in response to DNA double-strand breaks.
Burdova K*, Mihaljevic B*, Janscak P
Mol Cell, submitted

* equal contribution

Additional publications

Downregulation of Wip1 phosphatase modulates the cellular threshold of DNA damage signaling in mitosis.
Macurek L, Benada J, Müllers E, Halim VA, Krejčíková K, Burdová K, Pecháčková S, Hodný Z, Lindqvist A, Medema RH, Bartek J
Cell Cycle. 2013 Jan 15;12(2):251-62. doi: 10.4161/cc.23057.

Polo-like kinase 1 inhibits DNA damage response during mitosis.
Benada J, Burdová K, Lidak T, von Morgen P, Macurek L
Cell Cycle. Accepted for publication

B-Myb accumulates transiently at sites of DNA double-strand breaks and interacts with the Mre11-Rad50-Nbs1 complex.
Usadel C, Henrich S, Werwein E, Burdova K, Janscak P, Ferrari S, Klempnauer KH
Genes Dev, submitted