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Role of Rad18 in genome stability

Role Rad18 v kontrole stability genomu

Bachelor's thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 9. 5. 2019

Matouš Palek

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Abstract

Rad18 is an E3 ubiquitin ligase well-known for its function in DNA damage tolerance (DDT). Especially, its role in translesion DNA synthesis, one of two DDT branches, was extensively studied in the past. Recently, Rad18 was shown to be involved in the repair of DNA double-strand breaks (DSBs) in mammalian cells. The role of Rad18 in human cells seems to be important since DSB repair as well as DDT pathway are essential for maintenance of genome stability. In this work, I introduce the function of Rad18 in both DDT pathways, translesion DNA synthesis (TLS) and template switching (TS). Then I summarize current knowledge about the role of human Rad18 in DSB repair. Finally, I describe potential involvement of Rad18 dysregulation in human cancer, since loss of genome integrity is an important driving force for tumorigenesis.

Keywords: Rad18, genome stability, DNA double-strand break repair, tumorigenesis, DNA damage tolerance, translesion DNA synthesis, template switching.

Abstrakt

Rad18 je ubikvitin ligáza, která je známá pro svou funkci v dráze tolerance DNA poškození. V minulosti byla intenzivně studována především její účast v translézní DNA syntéze, což je jedna ze dvou drah umožňující toleranci DNA poškození. V nedávné době bylo ukázáno, že se Rad18 také účastní oprav dvouřetězcových zlomů DNA v savčích buňkách. Funkce proteinu Rad18 se tak jeví jako velice důležitá, neboť jak oprava dvouřetězcových DNA zlomů, tak tolerance DNA poškození jsou nepostradatelné pro zachování stability genomu. V této práci představuji funkci Rad18 v toleranci DNA poškození, které je zprostředkováno dvěma drahami, translézní DNA syntézou a replikací s dočasnou změnou templátu. Poté shrnuji současné poznatky o roli Rad18 v opravě dvouřetězcových zlomů. Nakonec se věnuji potenciálnímu přispění deregulace Rad18 k lidským nádorovým onemocněním, neboť ztráta integrity genomu je důležitý faktor umožňující tumorigenezi.

Klíčová slova: Rad18, stabilita genomu, oprava dvouřetězcových DNA zlomů, tumorigeneze, tolerance DNA poškození, translézní DNA syntéza, replikace s dočasnou změnou templátu.

Table of contents

1. Introduction 1

2. Biology and biochemistry of Rad18 2

2.1. Structure of Rad18 2

2.2. Function of Rad18 4

2.3. Regulation of Rad18 4

3. Functions of RAD18 in DNA damage tolerance pathway 6

3.1. Replication fork stalling 6

3.2. Translesion DNA synthesis 7

3.3. Template switching 9

4. RAD18 in double-strand break repair 12

4.1. DNA double-strand break repair pathways 12

4.2. DNA damage response and cell cycle arrest 15

4.3. Independent function of Rad18 in double-strand break repair and in DNA damage tolerance 15

4.4. Recruitment of Rad18 to double-strand breaks 16

4.5. Role of Rad18 in recruitment of homologous recombination repair factors 17

4.6. Role of Rad18 in ubiquitin signaling at double-strand breaks 18

4.7. Cell cycle dependent roles of Rad18 20

4.8. Role of Rad18 in checkpoint signaling 21

5. Potential role of Rad18 in cancer 23

5.1. Contribution of Rad18 role in translesion DNA synthesis 23

5.2. Contribution of Rad18 role in double-strand break repair 24

6. Conclusions 25

7. References 26

Abbreviations

53BP1	p53-binding protein 1
9-1-1	Rad9-Hus1-Rad1
A	alanine
Alt-EJ	alternative-NHEJ
APLF	aprataxin and PNK-like factor
ATM	ataxia telangiectasia, mutated
ATP	adenosine triphosphate
ATR	ataxia telangiectasia and Rad3-related
BRCA1	breast cancer 1
BRCT	BRCA1 C terminus
C	cysteine
Cdc25C	cell division cycle 25C
Cdk1	cyclin-dependent kinase 1
CPD	cyclobutane pyrimidine dimers
CPT	camptothecin
CtIP	carboxy-terminal binding protein-interacting protein
DDK	Dbf4/Drf1-dependent Cdc7 kinase
DDR	DNA damage response
DDT	DNA damage tolerance
DNA-PK	DNA-dependent protein kinase
DNA-PKcs	catalytic subunit of DNA-dependent protein kinase
DSB	DNA double-strand break
F	phenylalanine
FEN1	flap endonuclease 1
HJ	Holliday junctions
HLTF	helicase-like transcription factor
hPRC1L	human polycomb repressive complex 1-like
HR	homologous recombination
CHK1	checkpoint kinase 1

CHK2	checkpoint kinase 2
I	isoleucine
IR	ionizing radiation
IRIF	ionizing radiation-induced foci
JNK	ATR/Chk1-dependent c-Jun N- terminal kinase
K	lysine
kDa	kilodaltons
LRM	LR motif
MAGE-A4	melanoma antigen-A4
MCM	mini-chromosome maintenance
MDC1	mediator of DNA damage checkpoint protein 1
MRE11	meiotic recombination 11
MRN	MRE11-Rad50-NBS1
NBS1	Nijmegen breakage syndrome 1
NHEJ	non-homologous end joining
PCNA	proliferating nuclear antigen
PIP	PCNA-interacting peptide
pol η	DNA polymerase etha
pol ι	DNA polymerase iota
pol κ	DNA polymerase kappa
PTIP	PAX transcription activation domain interacting protein
Rad6BD	Rad6 binding domain
RAP80	receptor-associated protein 80
RIF1	RAP1 interacting factor
RING	really interested new gene
RNF168	ring finger protein 168
RNF8	ring finger protein 8
RPA	replication protein A
SAP	SAF-A/B, Acinus, Pias
SDSA	synthesis-dependent strand annealing

SHPRH	SNF2, histone-linker, PHD and RNF domain-containing helicase
SLF1	SMC5–SMC6 complex localization factor proteins 1
SLF2	SMC5–SMC6 complex localization factor proteins 2
SMC5	structural maintenance of chromosomes protein 5
SMC6	structural maintenance of chromosomes protein 6
SSA	single-strand annealing
ssDNA	single stranded DNA
TLS	translesion DNA synthesis
TS	template switching
UBZ	ubiquitin binding ZNF
USP1	ubiquitin specific protease 1
USP7	ubiquitin specific protease 7
UV	ultra violet radiation
XRCC4	X-ray repair cross-complementing protein 4
ZNF	C2HC zinc finger
ZRANB3	zinc finger, RAN-binding domain containing protein 3

1. Introduction

Cells are constantly challenged by intrinsic and exogenous DNA damage. This threat is constant and inevitable. The accumulation of DNA damage can lead to irreversible changes in genetic information and, in worst scenario, it can result in genomic instability and cellular death. In the course of evolution, cells have evolved various repair mechanisms to maintain genome integrity and faithfully transmit genetic information to next generations.

In general, DNA repair is initiated by sensor proteins that recognize damaged DNA. These molecular marks are responsible for subsequent recruitment of effector proteins that mediate repair of DNA lesion. However, the relationship between sensor and effector proteins is usually more complex. It is facilitated via an intricate network of mediators and transducers that direct and modulate the response. The choice of the appropriate pathway depends on the nature of DNA lesion and is crucial for faithful restoration of genome integrity. It is mediated by signaling based on various post-translational modifications such as phosphorylation, methylation, acetylation, poly(ADP-ribosylation) or modification by ubiquitin and ubiquitin-like modifiers. Many factors involved in these processes were recently identified revealing complexity and an extensive crosstalk among individual pathways. A good example of such protein is Rad18 that was found to be involved in ubiquitin signaling at various types of DNA lesions.

Rad18 plays a crucial role in DNA damage tolerance pathway that has been extensively studied on yeasts for several decades. Except this well-known role, Rad18 was shown to be involved in double-strand break repair. In this work, I give an overview of known roles of Rad18 ubiquitin ligase in human cells that are associated with maintenance of genome stability. I mainly focus on the recently described role of Rad18 in double-strand break repair. This function has not been yet fully understood. Therefore, I try to identify connections in published research and propose possible explanations for persistent contradictions. I also discuss potential involvement of Rad18 dysregulation in the loss of genome integrity as a basic characteristic of cancer.

2. Biology and biochemistry of Rad18

Rad18 was first described as a protein important for tolerance of yeasts to ultra violet radiation (UV) (reviewed in Prakash *et al.*, 1993). The protective effect of Rad18 was found to be dependent on E2 ubiquitin ligase Rad6. Both proteins were shown to directly interact in yeast and Rad18 was suggested to target Rad6 enzymatic activity to damage-containing regions in DNA (Sung *et al.*, 1994) Analogically, a human homolog of Rad18 interacts with Rad6 that is present in the human genome as two orthologues (Rad6A, Rad6B or UBE2A, UBE2B respectively). Together, they form a complex that possesses ubiquitin conjugating activity (Xin *et al.*, 2000). For clarity, I refer both Rad6 human homologs as Rad6 and Rad18 human homolog as Rad18.

2.1. Structure of Rad18

Rad18 is a conserved protein with several domains **(Figure 1)**. At its N-terminus, Rad18 harbors Really interested new gene (RING) domain that is common for majority of known ubiquitin E3 ligases and it is responsible for E2-enzyme binding (Tateishi *et al.*, 2000). Interestingly, RING domain of Rad18 alone is not sufficient for interaction with Rad6 but contribution of another domain is required. This domain is located in the C-terminal part of Rad18 spanning amino acid residues 340-395 and it is called Rad6 binding domain (Rad6BD) (Watanabe *et al.*, 2004).

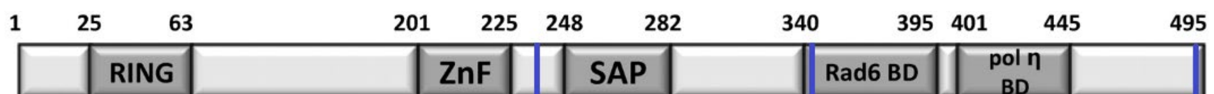

Figure 1. Secondary structure of human Rad18. Numbers indicate amino acid positions. The three putative nuclear localization signals are displayed as blue vertical lines. Adapted from Williams *et al.*, 2011.

The second function of RING domain is mediation of Rad18 homodimerization (Masuda *et al.*, 2012). This is not surprising since dimerization is common phenomena in E3 RING ligases that stimulates their activity (Deshaies & Joazeiro, 2009). Interestingly, Rad18 dimer asymmetrically interacts with only a single molecule of Rad6 suggesting a model where Rad6 binds RING and R6BD of the same Rad18 monomer (in cis) or it binds RING and R6BD of different monomers (in trans) **(Figure 2)** (Huang *et al.*, 2011).

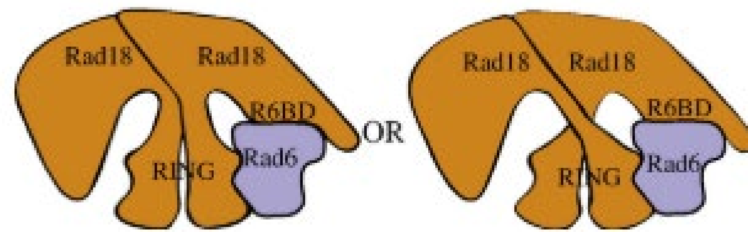

Figure 2. Structure of Rad18/Rad6 complex. The asymmetric dimerization of Rad18 ensures that only single molecule of Rad6 is bound and only single R6BD is occupied. Interaction in cis or trans is possible. Adapted from Huang *et al.*, 2011.

In addition, Rad18 contains C2HC zinc finger (ZNF) domain that was initially reported as a potential DNA binding domain and secondary homodimerization domain (Jones *et al.*, 1988; Miyase *et al.*, 2005). However, more recent data shows that ZNF domain is fully dispensable for both dimerization and enzymatic function of Rad18 (Huang *et al.*, 2011). Instead, ZNF domain was found to have ubiquitin binding ability (Notenboom *et al.*, 2007). And according its function, authors started referring it as a ubiquitin binding ZNF (UBZ) domain.

Next to UBZ, SAF-A/B, Acinus, Pias (SAP) domain is located and it was initially described as an DNA interacting domain (Notenboom *et al.*, 2007). However, in physiological salt concentration, its binding affinity is very low and probably insufficient for recruitment of Rad18 to ssDNA (Huttner & Ulrich, 2008). Although persisting contradictions, SAP domain is indispensable for Rad18 recruitment to sites of UV-induced DNA damage and for ubiquitination of target proteins (Nakajima *et al.*, 2006; Masuda *et al.*, 2012).

At the C-terminus, Rad18 possesses a motive that mediates interaction with DNA polymerase etha (Pol η) which is a crucial factor involved in translesion DNA synthesis **(see Section 3.2).**

Rad18 is localized predominantly in the nucleus. The localization pattern is diffuse, with few bright nuclear foci in G1 and nucleolar localization in late G2 (Inagaki *et al.*, 2009). In the secondary structure of Rad18, three putative nuclear localization signals were predicted that could be responsible for its import through nucleopore complex to the nucleus (Nakajima *et al.*, 2006).

2.2. Function of Rad18

As already mentioned, Rad18 is a member of RING domain E3 ubiquitin ligases that specifically interact with E2 ubiquitin conjugating enzyme Rad6. Rad6 possess intrinsic polyubiquitin chain-formation activity. Interaction with Rad18, however, restricts Rad6 to covalently attach only single ubiquitin residue to acceptor substrates (Hibbert *et al.*, 2011).

The most intensively studied and probably also the most important target for ubiquitin ligase activity of Rad18/Rad6 complex is proliferating nuclear antigen (PCNA), an essential factor involved in DNA replication. The ubiquitination occurs specifically at conserved lysine (K) 164 of PCNA in response to various types of DNA damage (Hoege *et al.*, 2002). The monoubiquitinated lysine then serves as a docking site for proteins involved in DNA damage tolerance pathway **(see Section 3)**.

Important factor antagonistic to Rad18 is ubiquitin specific protease 1 (USP1) that removes ubiquitin from PCNA facilitating flexible signaling at DNA damage sites (Niimi *et al.*, 2008).

Besides extensively studied E3 ubiquitin ligase activity, yeast Rad18 also possesses adenosine triphosphate (ATP) cleaving activity. The function of ATPase activity has not been determined yet, however, it was not detected in human Rad18 (Xin *et al.*, 2000).

2.3. Regulation of Rad18

Gene expression profiling revealed that Rad18 is expressed ubiquitously in various tissues in mammals (Masuyama *et al.*, 2005). Protein level is largely constant throughout the cell cycle and it remains the same even after DNA damage induction by UV. Interestingly, Rad18 mRNA levels fluctuate throughout the cell cycle with the minimum at G2 and peaking in S which suggests Rad18 translation is down-regulated in S-phase and up-regulated in G2-phase (Masuyama *et al.*, 2005). Similarly to other proteins involved in DNA damage repair, the function of Rad18 is tightly regulated in cells. This regulation occurs especially by post-translational modifications.

Estimated molecular weight of Rad18 is 63 kilodaltons (kDa), however, because of post-translational modifications it is detected as two species corresponding to 75 kDa and 85 kDa (Masuyama *et al.*, 2005; Notenboom *et al.*, 2007). The 85 kDa Rad18 is a monoubiquitinated form. This modification occurs through autoubiquitination and it seems to have a regulatory function. Non-ubiquitinated Rad18 is localized predominantly in the nucleus and it is sequestered to the cytoplasm upon autoubiquitination (Miyase *et al.*, 2005). Consistently, treatment with various DNA damaging agents leads to Rad18 deubiquitination by unknown

deubiquitinase. Moreover, inactivated ubiquitinated Rad18 molecules preferentially bind active non-ubiquitinated Rad18 species sequestering them and thereby inhibiting them in trans (Zeman *et al.*, 2014). This ubiquitin-based regulation demonstrates that more complicated regulation of Rad18 has evolved in higher eukaryotes, since it is absent in yeast (Miyase *et al.*, 2005). Ubiquitination also regulates Rad18 protein levels in cell, since K-48 linked polyubiubiquitination targets Rad18 to proteasomal degradation (Miyase *et al.*, 2005). This polyubiquitination is carried out by an unknown ubiquitin ligase and it is reversed by ubiquitin specific protease 7 (USP7) that cleaves polyubiquitin chains and thus stabilizes Rad18 (Zlatanou *et al.*, 2016).

Besides ubiquitination, also protein phosphorylation is involved in Rad18 regulation. Rad18 contains a conserved serine cluster at its C terminus that is phosphorylated in response to DNA damage. This modification is mediated by Dbf4/Drf1-dependent Cdc7 kinase (DDK) and ATR/Chk1-dependent c-Jun N- terminal kinase (JNK). Phosphorylated C terminal motif then serves as an docking site for binding partners of Rad18 (Day *et al.*, 2010; Barkley *et al.*, 2012).

3. Functions of RAD18 in DNA damage tolerance pathway

Accurate DNA replication is essential for maintaining genome stability. However, the progression of sensitive replication machinery can be impaired by intrinsic and extrinsic obstacles. As a result, it can lead to replication fork blockage and thus incomplete DNA replication or fork collapse, giving rise highly cytotoxic DSBs. To prevent that, cells employ the DDT, which facilitates continuation of replication. DDT is also sometimes termed as DNA post-replication repair, DNA damage avoidance, or replicative damage bypass, and it is not a repair pathway in the true sense of the term since it only enables bypass of damaged DNA template. The DNA lesion is subsequently repaired by some other repair pathway, usually by nucleotide excision repair. DDT is composed of two distinct pathways translesion DNA synthesis and template switching. Upon replication fork stalling, Rad18 facilitates downstream factors recruitment and subsequent initiation of either TLS or TS **(Figure 3)**.

3.1. Replication fork stalling

Replication forks are highly organized complexes of the template DNA, nascent DNA, and various regulatory and DNA processing proteins. In the front, Mini-chromosome maintenance (MCM) 2–7 helicase separates both DNA strands making them accessible for replicative DNA polymerases. Each strand is replicated by individual DNA polymerase that is bound by PCNA. PCNA is a polymerase processivity factor, that forms a doughnut-like homotrimeric complex, it encircles DNA and tightly holds DNA polymerase at the template.

As already mentioned, replication forks face numerous factors that can cause their slowing or even stalling. The malignant effect of such factors is termed replication stress. The most common source of replication stress are unrepaired DNA lesions such as bulky adducts caused by chemical mutagens, UV and by-products of cellular metabolism (reviewed in Zeman & Cimprich, 2014). UV-induced damage is a source of replication stress that activates DDT with high effectivity. Absorption of photons by DNA can initiate photochemical reactions resulting in formation of bulky lesions such as cyclobutane pyrimidine dimers (CPD) and thymine (6-4) photoproducts (reviewed in Ravanat *et al.*, 2001).

Bulky lesions are the most effective barriers for DNA polymerase. However, DDT can be also activated by types of replication stress that do not cause DNA damage. For example, it is effectively activated upon inhibition of replicative polymerase by aphidicolin or depletion of nucleotides by hydroxyurea treatment (Kannouche *et al.*, 2004; Lin *et al.*, 2011). Recent results

show, that DDT is also involved in tolerance to oncogene-induced DNA replication stress (Yang *et al.*, 2017).

Replicative fork stalling leads to uncoupling of DNA polymerase and MCM helicase activities. Although DNA polymerase is blocked, helicase continues unwinding DNA leading to accumulation of single stranded DNA (ssDNA) that is rapidly coated by replication protein A (RPA) (Byun *et al.*, 2005). RPA-coated ssDNA is essential for Rad18 recruitment to stalled forks. In this process, an interaction between RPA complex and Rad18 seems to be involved (Davies *et al.*, 2008; Hedglin *et al.*, 2019). Interestingly, also Nijmegen breakage syndrome 1 (NBS1) was shown to be important for PCNA ubiquitination and Rad18 recruitment. It directly interacts with Rad6BD of Rad18 via its Rad6-like domain. Authors of the study propose a model where asymmetric Rad18 dimer interacting with single Rad6 still has the other RAD6BD free for a simultaneous interaction with NBS1. Since NBS1 has RPA binding ability, it could promote Rad18 recruitment to RPA-coated ssDNA at stalled forks (Yanagihara *et al.*, 2011).

Thereafter, Rad18 must be chaperoned to the vicinity of its substrate, PCNA. RAD18 lacks any known PCNA-interacting motif, instead, the interaction between Rad18 and PCNA is believed to be indirect and probably mediated by Pol η. Pol η possesses interacting motives for both Rad18 and PCNA binding. And thus, it could target Rad18 to PCNA by physical bridging both proteins together (Kannouche *et al.*, 2004; Durando *et al.*, 2013). Recently, another protein, SIVA1, was proposed to have an analogical function (Han *et al.*, 2014).

3.2. Translesion DNA synthesis

PCNA monoubiquitinated at K164 serves as a docking site for TLS DNA polymerases that bypass DNA lesion and thus rescue stalled replicative fork making the lesion accessible to subsequent repair **(Figure 3)**. Based on phylogenetic relationships, eukaryotic TLS polymerases are classified into two families, Y and B **(Table 1)**. The more important Y family consists of Pol η, DNA polymerase iota (Pol ι), DNA polymerase kappa (Pol κ) and Rev1 (Prakash *et al.*, 2005). To specifically recognize monoubiquitnated PCNA, Y family polymerases are equipped with ubiquitin-binding domains and PCNA-interacting peptide (PIP) motives (Kannouche *et al.*, 2004; Bienko *et al.*, 2005).

Gene name	Protein name	Family	Characteristic
POLH/ XPV/RAD30A	Pol η	Y	Bypasses cyclobutane pyrimidine dimers relatively efficiently and accurately
POLK/DINB1	Pol κ	Y	Efficiently bypasses 2-deoxyguanosine lesions
REV1	REV1	Y	Specifically incorporates deoxycytidine monophosphate opposite deoxyguanosine and abasic sites
POLI/RAD30B	Pol ι	Y	Incorporates opposite polycyclic aromatic hydrocarbons adducts and lesions caused by oxidative damage
REV3L	REV3	B	Catalytic subunit of human DNA polymerase zeta (together with regulatory subunit Rev7)

Table 1. Overview of human translesion polymerases and their functions. Adapted from Goodman & Woodgate, 2013.

TLS polymerases possess more open active site compared to replicative polymerases resulting in less number of contacts to the template 5′ end. And thus, they can accommodate DNA template with bulky lesion. On the other hand, lower specificity is a trade-off that is necessarily connected with more open active site. (Trincao *et al.*, 2001) TLS polymerases incorporate wrong nucleotides with higher frequency than replicative polymerases. For example, Pol η has error rate 10^2 to 10^3 times higher than replicative DNA polymerase delta. (Washington *et al.*, 1999) On the other hand, replication of DNA lesions is relatively error-free if the appropriate TLS polymerase is used. For example, Pol η preferentially incorporates deoxyadenosines opposite the lesion rendering it perfectly suitable for thymine-thymine CPD bypass (Masutani *et al.*, 1999). And thus, recruitment of individual TLS polymerases must be tightly regulated. The importance of this process can be illustrated by a syndrome called xeroderma pigmentosum variant. Lack of Pol η results in error-prone CPD bypass by other TLS polymerases leading to increased mutagenesis and thus skin cancer propensity (McManus *et al.*, 2007).

3.3. Template switching

The single ubiquitin moiety bound on K164 can be further extended in K-63 linked polyubiquitin chain leading to replicative bypass by TS instead of TLS **(Figure 3)**. TS represents a model of error-free damage bypass in which the blocked nascent strand uses the undamaged sister chromatid as a template for replication. The bypass can occur through either a reversed fork intermediate or recombination-dependent strand invasion (reviewed in Branzei, 2011). The precise mechanism, however, is largely unknown.

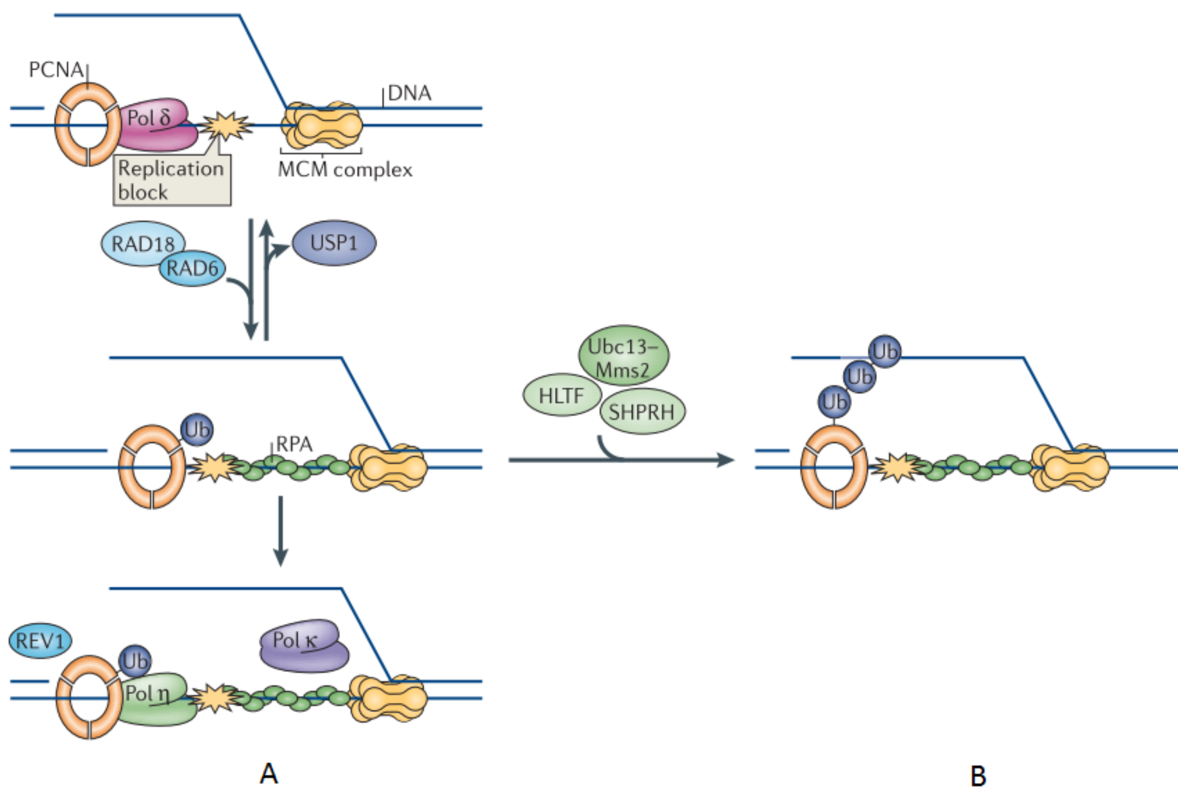

Figure 3. Role of Rad18 in DDT. Uncoupling of replicative polymerase and MCM helicase leads to PCNA ubiquitination by Rad18. This signal then enables polymerase switching and lesion bypass by TLS polymerases (A). Alternatively, PCNA can be further polyubiquitinated which leads to error-free TS (B). Adapted from Mailand *et al.*, 2013.

In yeast, the polyubiquitination of PCNA is performed by heterodimeric E2 ubiquitin-conjugating enzyme UBC13-MMS2 in cooperation with E3 ubiquitin-ligase Rad5 (Ulrich & Jentsch, 2000; Hoege *et al.*, 2002). Although PCNA polyubiquitination is mediated by Rad5, Rad18 is indispensable for this process (Hoege *et al.*, 2002). Firstly, Rad18-mediated PCNA monoubiquitination has priming function for subsequent ubiquitin chain formation and

secondly, Rad18 facilitate recruitment of Mms2–Ubc13-Rad5 complex to stalled fork by direct interaction with Rad5 (Ulrich & Jentsch, 2000).

In mammalian cells, two Rad5 orthologs, helicase-like transcription factor (HLTF) and SNF2, histone-linker, PHD and RING finger domain-containing helicase (SHPRH), have been identified. Both of them were shown to polyubiquitinate PCNA in vitro (Unk *et al.*, 2006, 2008). However, their role in vivo is questionable since HTLF/SHPRH double knockout murine cells are still capable of PCNA polyubiquitination and do not show increased sensitivity to DNA damage (Krijger *et al.*, 2011).

Although PCNA polyubiquitination is readily observed after DNA damage in yeast, it is hard to detect in mammalian cells (Kannouche *et al.*, 2004). Moreover, it was proposed that DDT of higher eukaryotes could be completely independent on PCNA polyubiquitination (Gervai *et al.*, 2017).

Regardless of persistent contradictions, the current model of TS does include PCNA ubiquitination **(Figure 4)**. Polyubiquitinated PCNA is directly recognized by zinc finger, RAN-binding domain containing 3 (ZRANB3) (Weston *et al.*, 2012). ZRANB3 possess canonical PIP box and NPL4 zinc finger motif that specifically recognizes K63-linked ubiquitin chains. Bound to PCNA, it promotes fork restart via fork reversal (Vujanovic *et al.*, 2017). Alternatively, structure-specific endonuclease activity of ZRANB3 induces a DNA break and promotes fork reversal to stabilize the fork. The 5' end of DNA strand containing lesion is then cleaved by flap endonuclease 1 (FEN1), while the free 3' end is extended by DNA polymerase (Weston *et al.*, 2012).

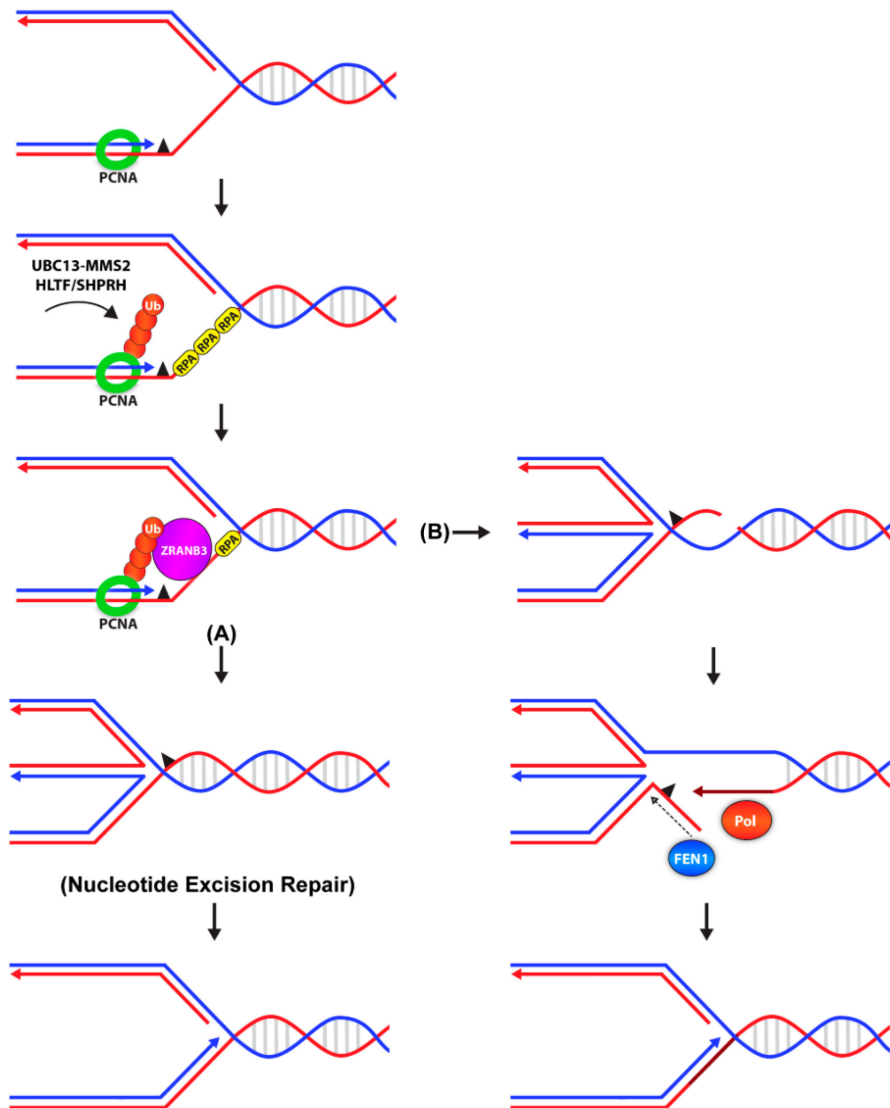

Figure 4. Role of ZRANB3 at stalled replication fork. ZRANB3 promotes error free lesion bypass by fork reversal (A) or it mediates direct repair of DNA lesion (B). Adapted from Leung *et al.*, 2019.

4. RAD18 in double-strand break repair

DSBs in the DNA backbone are highly cytotoxic DNA lesions directly threatening chromosomal integrity. This type of DNA damage can be caused by various factors such as ionizing radiation, reactive oxygen species, failures of DNA replication or physiological cleavage by DNA processing enzymes. Although DSBs have different origin, the repair is the same.

Pathways responding to DSBs-inducing DNA damage are endowed with remarkable robusticity that is facilitated by high number of involved proteins. Many of such factors have been recently identified revealing surprising complexity of these processes and an extensive crosstalk with other DNA repair pathways.

Rad18 is best known for its crucial function in PRR. However, emerging evidence strongly suggests that Rad18 also plays an important role in DSB repair, although the precise mechanism is still elusive.

4.1. DNA double-strand break repair pathways

Eukaryotic cells employ two major pathways, non-homologous end joining (NHEJ) and homologous recombination (HR), to repair DSBs. However, in some cases other alternative pathways may be used, such as single-strand annealing (SSA) and alternative-NHEJ (Alt-EJ) (also known as microhomology-mediated end joining) (reviewed in Ceccaldi *et al.*, 2016).

During NHEJ, Ku heterodimer (Ku70 and Ku80) directly binds to both ends of the DSB bringing them together (Walker *et al.*, 2001). Ku subsequently recruits and activates the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) that stabilizes DSB ends and prevents their resection by HR nucleases. DSBs containing damaged and thus non-ligable ends must be processed prior ligation by various enzymes such Artemis and aprataxin and PNK-like factor (APLF). Finally, X-ray repair cross-complementing protein 4 (XRCC4) and DNA ligase IV are loaded to carry out DNA ends relegation (reviewed in Ciccia & Elledge, 2010). Repair by NHEJ is fast and it can operate throughout the cell cycle. Nevertheless, NHEJ-mediated blunt-end ligation is independent of sequence homology and thus it is potentially mutagenic.

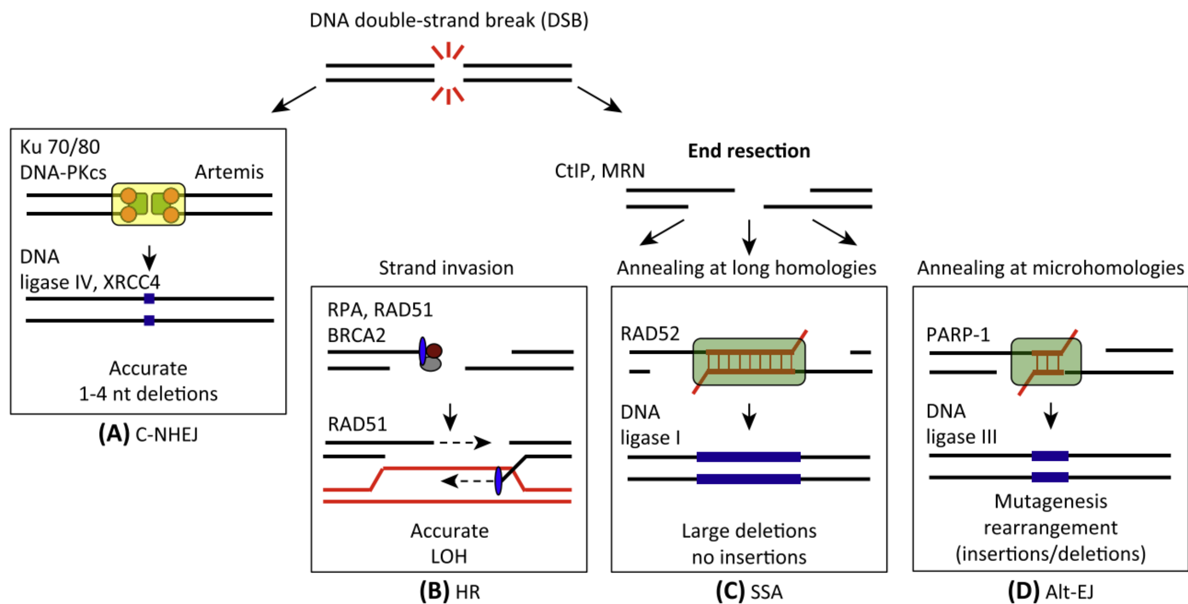

Figure 5. DSBs repair pathways. The pathway choice depends on the extent of DNA end processing. Blunt ends can be directly ligated by NHEJ (A). Their resection, however facilitates repair based on complementarity. Besides accurate HR (B), resected DNA end can be directly annealed to each other at large or short homologies leading to SSA (C) and Alt-EJ (D) respectively. Adapted from Ceccaldi *et al.*, 2016.

In contrast to NHEJ, HR is solely restricted to S and G2 phases of the cell cycle since it is dependent on the availability of sister chromatid that serves as a template for homology directed repair. At the beginning, DSB is recognized by meiotic recombination 11 (MRE11) - Rad50-NBS1 complex (MRN), which together with carboxy-terminal binding protein-interacting protein (CtIP) initiates resection of DNA ends (Sartori *et al.*, 2007; Williams *et al.*, 2007). This process leads to 3′ single-strand DNA overhangs formation that are rapidly coated by ssDNA binding protein RPA (reviewed in Ciccia & Elledge, 2010). Finally, RPA is removed and replaced by Rad51 that mediates strand invasion and annealing with the undamaged strand of sister chromatid **(Figure 6)**. DNA strand that invades sister chromatid forming D-loop and it is further extended in a process called synthesis-dependent strand annealing (SDSA). D-loop subsequently dissociates and a gap in the second strand is filled and both DNA strands are ligated. Alternatively, double Holliday junctions (HJ) can be formed after ligation of the invading strand with the second strand. HJ are then dissolved resulting in either crossover or noncrossover (reviewed in San Filippo *et al.*, 2008).

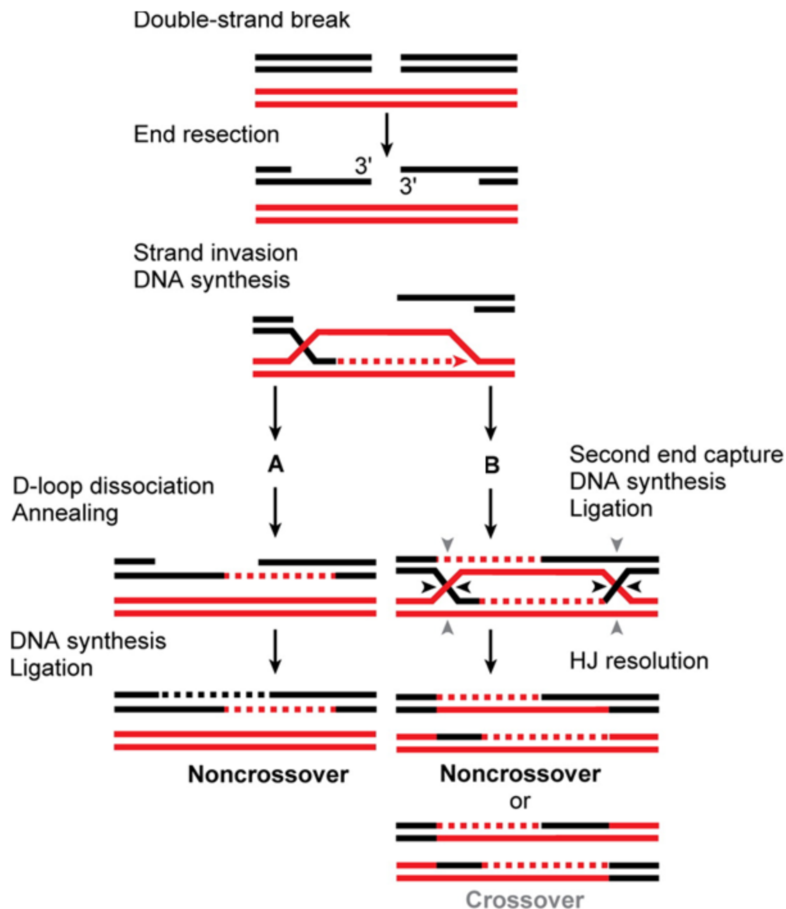

Figure 6. Mechanism of repair by homologous recombination. It can occur either by synthesis-dependent strand annealing (A) or by recombination via double HJ (B). Adapted from San Filippo *et al.*, 2008.

In G0/G1, cells have to rely on error-prone NHEJ. However, in S/G2, when duplicated chromatids are available, also HR becomes active. And thus, DSBs are sites of competition between HR and NHEJ. The pathway choice is determined by resection of DNA ends that is tightly regulated. This regulation involves cyclin-dependent kinases that through phosphorylation activate involved nucleases (Huertas & Jackason, 2009). Another regulatory level includes p53-binding protein 1 (53BP1) and breast cancer 1 (BRCA1). 53BP1 recruits downstream effectors RAP1 interacting factor (RIF1) and PAX transcription activation domain interacting protein (PTIP) leading to stabilization of NHEJ proteins at DSB ends. On the other hand, antagonistically acting BRCA1 removes 53BP1 from DSBs to facilitate resection and Rad51 loading (reviewed in Daley & Sung, 2014).

4.2. DNA damage response and cell cycle arrest

DNA damage response (DDR) is a protective pathway that senses various types of DNA lesions and mediates a complex response that involves DNA repair, cell cycle checkpoints activation, apoptosis and senescence pathways. DDR is initiated by signaling of kinases from phosphatidylinositol 3-kinase-related family – ataxia telangiectasia, mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK) that are recruited and activated by DNA damage sensor proteins (reviewed in Sirbu & Cortez, 2013).

Subsequently, downstream kinases checkpoint kinase 1 (CHK1) and checkpoint kinase 2 (CHK2) are phosphorylated. Together, ATM/ATR and CHK1/CHK2 then phosphorylates hundreds of effector proteins that have wide spectrum of functions (reviewed in Ciccia & Elledge, 2010).

The cell cycle arrest is then primarily mediated by phosphorylation of cell division cycle 25C (Cdc25C) by activated CHK1 and CHK2 (Matsuoka *et al.*, 1998; Sanchez *et al.*, 2008). Since Phosphorylated Cdc25C is unable to activate cyclin-dependent kinase 1 (Cdk1) that is required for mitotic entry (Peng *et al.*, 1997). The cell cycle arrest is a crucial step that allows cells to finish DNA repair before entering mitosis. And thus, it prevents transferring of damaged DNA to daughter cells.

DSBs are highly cytotoxic and they effectively activate DDR. In response to DSB-inducing damage, ATM phosphorylates histone from H2AX subfamily at serine 139 in chromatin surrounding DSB (Rogakou *et al.*, 1998). Phosphorylated H2AX (γH2AX) then serves as a docking site for mediator of DNA damage checkpoint protein 1 (MDC1) that is responsible for recruitment of ring finger protein 8 (RNF8) ubiquitin ligase (Mailand *et al.*, 2007). Moreover, γH2AX promotes ATM accumulation via positive feedback loop (Falck *et al.*, 2005).

4.3. Independent function of Rad18 in double-strand break repair and in DNA damage tolerance

It was repeatedly demonstrated that Rad18 deficient mammalian cells are sensitive to DSB-inducing agents such as ionizing radiation (IR) and camptothecin (CPT) (Saberi *et al.*, 2007; Huang *et al.*, 2009). Moreover, Rad18 accumulates at ionizing radiation-induced foci (IRIF) and colocalize with well-known DSB markers Rad51 and γH2AX suggesting a direct role in repair pathway (Huang *et al.*, 2009). This function has probably developed later in the course of evolution since yeast Rad18 mutants do not show defect in DSB repair (Saberi *et al.*, 2007).

Survival assays show that only ZNF and RING domains of Rad18 are essential to restore resistance to IR and CPT (Huang *et al.*, 2009). As previously discussed, RING domain is connected to E3 ligase function of Rad18. However, Rad18 with single amino acid substitution inside RING incapable to ubiquitinate PCNA still fully rescues phenotype of wild type protein suggesting E3 ligase activity is not required for cell survival after IR induced DNA damage (Huang *et al.*, 2009).

Interestingly, Rad18 is recruited to DSBs independently on PCNA (Inagaki *et al.*, 2009) that indicates the role of Rad18 at DSBs is separable to its role in TLS. Indeed, IR fails to induce PCNA monoubiquitination and cells with mutated K164 of PCNA are not increasingly sensitive to CPT (Saberi *et al.*, 2007). Consistently, ZNF domain is dispensable for both PCNA ubiquitination and UV tolerance (Huang *et al.*, 2009). These findings are rather surprising since ZNF domain-dependent function of Rad18 in DSB repair is present exclusively in higher eukaryotes. ZNF domain, however, is conserved from yeast.

4.4. Recruitment of Rad18 to double-strand breaks

Mammalian cells treated with DSB inducing agents rapidly recruit Rad18 to sites of DNA damage (Inagaki *et al.*, 2009). Concomitantly, biochemical fractionation experiments showed that Rad18 becomes enriched on chromatin after such treatment (Huang *et al.*, 2009).

As already mentioned, Rad18 interacts with NBS1, a part of MRN complex responsible for DSB detection. However, Rad18 is recruited to IRIF even in NBS1 deficient cells (Huang *et al.*, 2009). Instead, screen for other DNA repair factors revealed Rad18 foci formation depends on H2AX, MDC1 and RNF8 which indicates that Rad18 acts downstream these factors in the known DNA damage signaling cascade. Specifically, the accumulation of Rad18 at DSBs requires RNF8 ligase activity which is supported by the fact that foci formation can be impaired by the depletion of free ubiquitin after a treatment with a proteasome inhibitor (Huang *et al.*, 2009).

In comparison to proteins that bind DSB lesion itself such as Rad51 and RPA, Rad18 position covers larger area indicating it is bound to surrounding chromatin (Inagaki *et al.*, 2009). For recruitment to IRIF only ZNF domain of Rad18 is indispensable and as already mentioned, this domain possess ubiquitin binding ability (Huang *et al.*, 2009). And since IRIFs are sites of extensive ubiquitination facilitated by RNF8 and ring finger protein 168 (RNF168), all these findings strongly support the idea that Rad18 directly binds ubiquitinated chromatin components surrounding DSB **(Figure 7)**.

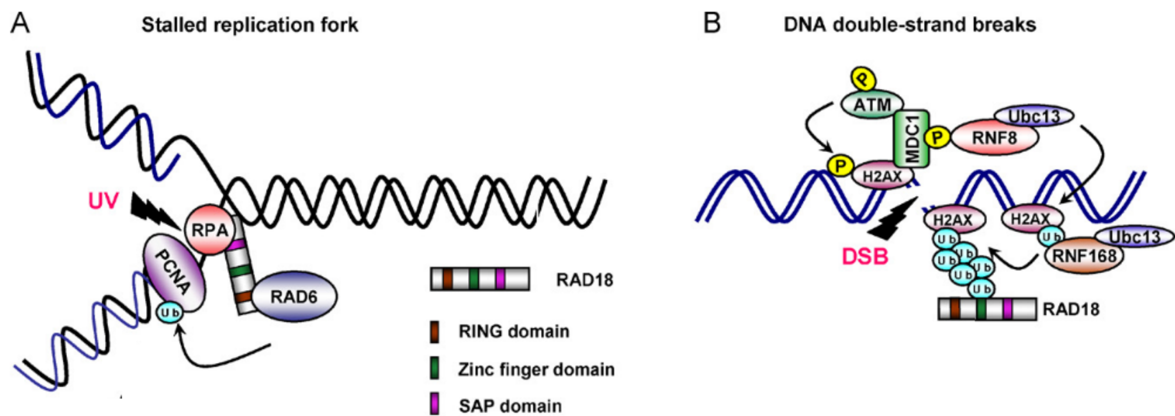

Figure 7. Recruitment of Rad18 to different DNA lesions. At stalled replication fork, Rad18 is recruited by RPA that is accumulated at ssDNA (A). At DSBs, Rad18 UBZ domain binds chromatin components such as H2AX ubiquitinated by RNF8 and RNF168 (B). Adapted from Ting *et al.*, 2010.

4.5. Role of Rad18 in recruitment of homologous recombination repair factors

Mass spectrometry analysis identified Rad51C as a Rad18 interacting protein (Huang *et al.*, 2009). Rad51C is one of the five Rad51 paralogs that are implicated in HR repair in mammalian cells. Rad51C interacts with Rad51 and promotes its accumulation at DSBs (Takata *et al.*, 2002; Rodrigue *et al.*, 2006) The interaction with Rad18 was shown to facilitate the retention of Rad51C to chromatin after DNA damage. Consistently, impaired function of Rad51C in Rad18 deficient cells leads to improper Rad51 foci formation. The interaction with Rad51C occurs via RING domain of Rad18, independently on E3 ubiquitin-ligase activity, indicating Rad18 plays the role of an adapter sequestering Rad51C at DSB sites (Huang *et al.*, 2009). This idea of Rad18 as a simple adapter is supported by the fact that the role of Rad18 in HR promotion is strictly dependent on its ability to localize at sites of DSBs.

Another potential role of Rad18 is recruitment of structural maintenance of chromosomes proteins 5 and 6 (SMC5 and SMC6) to DSBs. These two proteins are important for recruitment of cohesin complex that is responsible for maintaining the close proximity of sister chromatid during HR (Potts *et al.*, 2006).

Rad18 was shown to interact with SMC5–SMC6 complex indirectly via two adaptor proteins SMC5–SMC6 complex localization factor proteins 1 and 2 (SLF1 and SLF2). The organization of whole complex is linear, and it is independent on Rad6. SMC5-SMC6 is bound to SLF1 via

SLF2, and SLF1 interacts through BRCA1 C-terminus (BRCT) domain with phosphorylated residues 424 and 422 of Rad18 (Liu *et al.*, 2012; Räschle *et al.*, 2015) **(Figure 8)**. Interestingly, this function seems to be dispensable since cells with abolished SLF1 show no significant sensitivity to DNA damaging agents (Ng *et al.*, 2005).

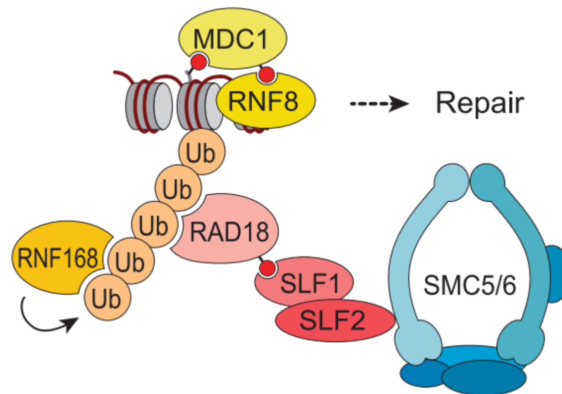

Figure 8. Model of the SMC5/6 recruitment to damaged DNA. Rad18 bound on ubiquitinated chromatin recruits the SMC5/6 complex via two adaptor proteins, SLF1 and SLF2. Adapted form Räschle *et al.*, 2015.

4.6.Role of Rad18 in ubiquitin signaling at double-strand breaks

Rad18 is recruited to DSBs by its ability to bind ubiquitinated chromatin, as discussed in previous sections. However, the ubiquitin signaling network is intriguingly complex including poly-/mono-/multi-ubiquitination of various proteins such histones H1, H2A/H2AX, and H2B (reviewed in Schwertman *et al.*, 2016). Which of these modifications are responsible for Rad18 recruitment is not yet clear. Pull down experiments showed that Rad18 binds large pool of ubiquitinated proteins and this binding does not seem to be promiscuous but rather specific. Further analysis identified monoubiquitinated histone H2A at K119 as a Rad18 binding partner (Inagaki *et al.*, 2011). However, this interaction alone cannot explain accumulation of Rad18 at DSB. Since, as will be discussed later, Rad18 has higher affinity to polyubiquitin chains compared to ubiquitin monomers implicating that polyubiquitinated proteins are more attractive candidates for Rad18-binding partners. Moreover, the interaction is not enhanced after IR induced damage corresponding to the fact that ubiquitination of histone H2A at K119 has no known role in DNA damage signaling and it is RNF8/RNF168 independent. Instead, this H2A modification is known to play a role in transcriptional gene silencing and to be mediated by human Polycomb repressive complex 1-like (hPRC1L) (Wang *et al.*, 2004).

Mutagenic analysis showed that UBZ alone is not sufficient for Rad18 recruitment to IRIF. Only UBZ extended with 16 residues at its C-terminus forms foci suggesting that ubiquitin binding module of Rad18 has bipartite structure composed of UBZ core sequence and proximal short peptide motif that provide specificity. This short targeting peptide is termed LR motif (LRM). Such bipartite architecture is not unusual, it can be found also in other polyubiquitin binding proteins for example in RNF168 and receptor-associated protein 80 (RAP80) (Panier *et al.*, 2012).

Rad18 UBZ binds ubiquitin monomers and oligomers with similar affinities. However, extension of UBZ with LRM provide whole domain specificity to bind K-48 polyubiquitin and especially K-63-linked polyubiquitin with high affinity rather than monomeric ubiquitin. According the current model, UBZ binds a single ubiquitin moiety and LRM mediates contact with the proximal ubiquitin moiety endowing Rad18 with ability to specifically recognize polyubiquitin chains (Thach *et al.*, 2015). Molecular modeling reveals that UBZ and LRM form single continuous helix and the affinity of such continuous binding domain to ubiquitin chains is surprisingly high (Thach *et al.*, 2015). As a result, massive Rad18 overexpression interfere with IR induced recruitment of other ubiquitin-binding DDR proteins such as 53BP1, RAP80, RNF168 and BRCA1 suggesting a competition for the same binding site. (Helchowski *et al.*, 2013) Based on that, overexpressed Rad18 interferes with ubiquitin signaling at DSBs by two ways. Firstly, it blocks existing polyubiquitinated chromatin tags making them inaccessible for other proteins and secondly, it impedes further spreading of ubiquitin modifications across chromatin by interfering with positive feedback loop of RNF168.

In vitro, Rad18 strongly interacts with histone H2A ubiquitynated at K15 in the nucleosome core particle dimethylated at histone H4 K20 (Hu *et al.*, 2001). This pattern of nucleosome modifications is induced by DNA damage and is specifically crucial for 53BP1 recruitment. Interestingly, the binding affinity of Rad18 is about two orders of magnitude higher than that of 53BP1 for the same substrate (Hu *et al.*, 2001). Rad18 is therefore potential strong competitor to 53BP1.

This behavior is reminiscent of RNF169, a recently characterized E3 ubiquitin ligase paralogous to RNF168. RNF169 binds chromatin ubiquitinated at DSBs. However, in contrast to RNF168, it does not participate on chromatin modification (Poulsen *et al.*, 2012). Rad18 and Rad169, having similar affinities to ubiquitinated histone H2A K15 (Hu *et al.*, 2001), could be employed in repair pathway decision independently on their catalytical activity. According this

attractive hypothesis, they would facilitate end resection and HR repair by inhibiting 53BP recruitment to DSBs.

As already mentioned ectopically expressed Rad18 abolishes 53BP1 recruitment to IRIF. However, it is important to be aware that this phenotype was observed upon high Rad18 overexpression and thus, to which extent is this function relevant in physiological conditions remains open to discussion. In agreement with suggested scenario, both Rad18 and RNF196 depleted cells have decreased HR efficiency (Huang *et al.*, 2009; Poulsen *et al.*, 2012). However, it is not clear whether it is caused solely by unrestricted function of 53BP1. In fact, decision between NHEJ and HR depends on multiple factors, and thus, the situation may be more complex.

4.7.Cell cycle dependent roles of Rad18

As discussed above, Rad18 seems to promote DSB repair by HR. However, although HR is active only in S/G2 phases, Rad18 is recruited to IRIF independently on cell cycle (Inagaki *et al.*, 2009). Yet, the mechanism of recruitment does not seem to be the same. In S/G2, Rad18 is recruited conventionally by interaction with K64 polyubiquitin chains to DSBs. In G1, however, Rad18 was reported to be recruited by direct interaction with 53BP1 (Watanabe *et al.*, 2009).

Watanabe et al. suggest a model where Rad18 binds 53BP1 via UBZ domain specifically in G1. Subsequently, Rad18 monoubiquitinates K1268 of 53BP1 promoting its stability at DSB. Although being very attractive, this model still lacks necessary experimental support. Although Rad18 was shown to monoubiquitinate 53BP1 in vitro, the modification was not detected in cells. In addition, this study tries to reproduce phenotype of Rad18 loss by mutation of K1268 of 53BP1. But such intervention has more complex impact on 53BP1 function since K1268 was recently shown to be target of ubiquitination activity of RNF168 (Bohgaki *et al.*, 2013).

In addition, this model requires Rad18 E3 ubiquitin activity. However, there is no clear consensus about the importance of this activity in DSB repair (Yamazoe *et al.*, 2006; Palle & Vaziri, 2011; Huang *et al.*, 2009). The contradiction in published results could be caused by the inappropriate experimental setups. Depletion of Rad6 does not abolish activity of Rad18 exclusively since Rad6 was shown to cooperate with RNF8 during DSB repair (Keller *et al.*, 2013). Also single amino acid mutants of RING domain C28F and I27A used in studies are not appropriate since I27A was shown to have residual enzymatic activity and C28F has seriously disrupted structure and nuclear localization (Tateishi *et al.*, 2000; Helchowski *et al.*, 2013).

Taken together, study of Watanabe et al. suggests an attractive model where Rad18 functions as a negative regulator of 53BP1 in G2/S (thereby inhibiting NHEJ) and as a positive regulator in G1 (thereby facilitating NHEJ). Regardless persisting doubts, this model could explain sensitivity of avian DT40 Rad18 deficient cells to IR that is surprisingly increased in G1. In addition, the direct role of Rad18 in NHEJ is further supported by the fact, that it colocalizes with Ku positive IRIFs that represent non-resected DSBs being repaired by NHEJ (Inagaki *et al.*, 2009).

4.8. Role of Rad18 in checkpoint signaling

It was shown that mammalian cells with abolished Rad18 have abrogated cell cycle progression. Specifically, Rad18 deficient cells fail to activate G2/M checkpoint in response to IR and proceed to M phase resulting in loss of genome integrity manifested by micronuclei formation. Moreover, these cells have reduced level of p53 phosphorylated at serine 15 and ATM phosphorylated at serine 1981 following IR (Sasatani *et al.*, 2015). These changes indicate decreased DDR signaling, since autophosphorylation at serine 1981 is a marker of activated ATM and p53 is a target of both ATM and ATR.

Important component of checkpoint signaling is Rad9-Hus1-Rad1 (9-1-1) complex. In response to some types of DNA damage, 9-1-1 complex is loaded on DNA and it is thought to promote activation of checkpoint kinases ATR and Chk1 (reviewed in Parrilla-Castellar *et al.*, 2004). 9-1-1 complex consists of three different subunits and its structural similarity to doughnut-shaped PCNA suggests an attractive hypothesis that it could be a target of Rad18.

In mammalian cells, Rad18 was shown to be important for recruitment of Rad1 to damaged chromatin. In this process, Rad18 acts probably indirectly since interaction between these proteins was not detected. Moreover, their localization pattern at IRIF is different. While Rad9 forms discrete foci, Rad18 shows diffused localization surrounding these foci (Inagaki *et al.*, 2011).

The connection between 9-1-1 complex and Rad18 was examined in more details in yeast. The yeast 9-1-1 complex consists of corresponding orthologs Rad17, Ddc1 and Mec3. Similarly to mammalian cells, also Rad18 deficient yeast cells show disrupted checkpoint signaling (Verkade *et al.*, 2013). And moreover, Rad18 was shown to interact with Rad17 (Yeung *et al.*, 2008).

Initially, yeast Rad17 was reported to be ubiquitinated in response to DNA damage in a Rad6/Rad18-dependent manner. The search for potential site of this modification based on

sequence alignment revealed K197 of Rad17 sharing homology with essential K164 of PCNA. Consistently, Rad17 with mutated K197 failed to be ubiquitinated by Rad18 after treatment with alkylating agents (Yeung *et al.*, 2008). However, more recent study gives an evidence that ubiquitination of 9-1-1 complex is Rad18 independent and unimportant for the DNA damage response (Davies *et al.*, 2010). Moreover, the crystal structure of 9-1-1 complex showed that K197 is situated on the opposite side of the clamp compared to K164 in PCNA. Structure-based alignment revealed that rather K185 than K197 of Rad1 is topologically similar to K164 of PCNA (Doré *et al.*, 2009). However, neither K185 seems to be involved in regulation of the complex (Wit *et al.*, 2011).

5. Potential role of Rad18 in cancer

Genome instability and mutagenesis are important enabling characteristics of cancer that drive tumor progression (Hanahan & Weinberg, 2011). And thus, defects in factors involved in DNA repair are commonly found in cancer cells and mutations of these proteins can display cancer predisposition (Hoeijmakers, 2001).

Also Rad18, as a protein important for genome integrity maintenance, could be potentially involved in carcinogenesis **(Figure 9)**. A survey of The Cancer Genome Atlas revealed amplifications or inactivating deletions in both Rad18 and Rad6 genes in several cancer types. For example, 11% of renal cell carcinoma and 5% of pancreatic tumors have deletions of Rad18 gene (Buoninfante *et al.*, 2018). In addition, Rad18 was shown to be stabilized by melanoma antigen-A4 (MAGE-A4) that is aberrantly expressed in various cancers (Gao *et al.*, 2016). The fact that Rad18 performs various roles in DNA repair raises a question which of them could be relevant in human cancer.

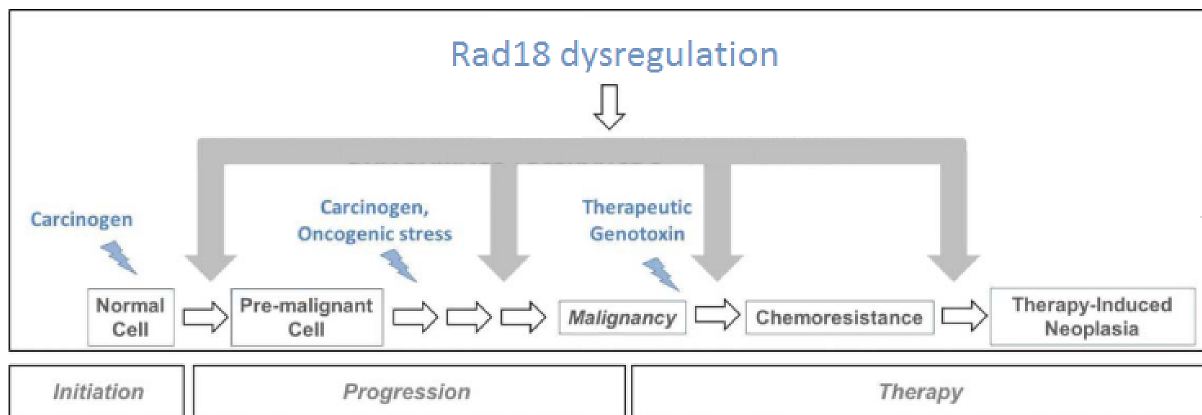

Figure 9. **Potential roles of Rad18 in tumorigenesis.** Mutagenesis mediated by dysregulated TLS can promote malignant transformation. The protective capacity of Rad18-mediated repair, on the other hand, helps cancer cells to overcome DNA damage caused by oncogenic stress and eventually it can mediate chemoresistance. Adapted from Mutter-Rottmayer *et al.*, 2016.

5.1. Contribution of Rad18 role in translesion DNA synthesis

Rad18, a key regulator of TLS, could play a role in tumorigenesis since both TLS downregulation or upregulation can threaten genome integrity. Reduced TLS activity result in increased cellular sensitivity to DNA damage. On the other hand, overactivation and of intrinsically error-prone TLS leads to increased mutagenesis.

As already mentioned, skin cancer in XPV patients is a well-documented case of cancer caused by aberrant TLS. However, emerging evidence suggests that TLS dysfunction contribute to various human cancers. Analysis of genome sequencing data revealed at least 30 mutational signatures commonly found in human cancers out of which two could be attributed to TLS polymerases (Alexandrov *et al.*, 2013). Besides skin cancer, Pol η mutational signatures were identified also in other types of cancer such as lung, ovary, bladder, breast, and prostate cancer (Rogozin *et al.*, 2018). In addition, TLS polymerases were shown to be overexpressed in range of tumor types (Albertella *et al.*, 2005b).

Although error-prone, the repair capacity of TLS polymerases could help cancer cells to cope with increased level of DNA damage. Pol κ was shown to be important for resistance to replication stress induced by oncogene signaling (Yang *et al.*, 2017). And Pol η was shown to mediate tolerance to damage induced by cisplatin, an anticancer drug causing intrastrand cross-linking adducts (Albertella *et al.*, 2005a).

5.2.Contribution of Rad18 role in double-strand break repair

Dysregulation of Rad18 in DSB repair might contribute to genome instability in cancer cells. As already discussed, Rad18 loss result in reduced DSB repair. Elevated Rad18 expression, on the other hand, limits DSB repair as well, since ubiquitin signaling at DSBs becomes impaired (Helchowski *et al.*, 2013). These findings support the double-edged sword nature of Rad18 dysregulation.

In addition, engagement of Rad18 in DSB repair could be connected to therapeutic response of cancer cells. Accordingly, expression of RAD18 in glioma cells corresponds to their radioresistance (Xie *et al.*, 2014). And Rad18 was shown to be important for resistance to PARP inhibitors that are commonly used in chemotherapy for cancers with defects in DSB repair (Saberi *et al.*, 2007).

6. Conclusions

About three decades of intensive research helped us to understand the role of Rad18 in signaling at replication fork. In addition, the role of Rad18 in DSB repair has recently been unveiled. To date, it is clear that function of Rad18 in TLS is fully separable from its function in DSB repair. In fact, the differences are striking. The role of Rad18 in TLS is completely dependent on its E3 ubiquitin ligase activity. In DSB repair, on the contrary, Rad18 seems to act as a simple adaptor or competitive inhibitor. In addition, proteins interacting with Rad18 that are crucial for TLS appear not to be involved in DSB repair. The recruitment of Rad18 to DSBs depends on its UBZ domain and ubiquitinated chromatin while in TLS, UBZ domain seems to be completely redundant and Rad18 is recruited by RPA.

Although we have essential knowledge about involvement of Rad18 in DSB repair, the overall picture is blurry, and many questions remain unanswered. Further research is needed to verify still very attractive hypothesis, that ubiquitin E3 ligating activity of Rad18 has its targets at DSBs. Also the role of Rad18 in HR promotion is not clear. What aspect is more important, does Rad18 promote HR directly by recruitment of downstream proteins, or does it act indirectly by blocking 53BP1 function and thus it tips the scales in favor of HR? In addition, it is not clear how Rad18 could simultaneously promote such contradictory pathways as HR and NHEJ.

Although the precise mechanism is not yet understood, both pathways, DDT and DSB repair, are crucial for maintenance of genome integrity, and Rad18 seems to possess a characteristic of a double-edged sword guarding the genome. Both downregulation and upregulation of Rad18 disrupt proper function of TLS and DSB repair to become a sources of genome instability. Based on that, Rad18 has full potential to facilitate mutagenesis and genome instability that enable carcinogenesis. However, we lack necessary evidence to assess whether this potential is manifested in human cancers. Although therapeutic significance is not clear, Rad18 dysregulation could potentially serve as a prognostic marker for human cancer and it could be useful in prediction of a therapeutic response to therapy.

7. References

Albertella MR, Green CM, Lehmann AR, O'Connor MJ. **2005a**. A role for polymerase η in the cellular tolerance to cisplatin-induced damage. *Cancer Research* **65**: 9799–9806.

Albertella MR, Lau A, O'Connor MJ. **2005b**. The overexpression of specialized DNA polymerases in cancer. *DNA Repair* **4**: 583–593.

Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin A V., Bignell GR, Bolli N, Borg A, Børresen-Dale, *et al.* 2013. Signatures of mutational processes in human cancer. *Nature* **502**: 415–424.

Barkley LR, Palle K, Durando M, Day TA, Gurkar A, Kakusho N, Li J, Masai H, Vaziri C. **2012**. c-Jun N-terminal kinase-mediated Rad18 phosphorylation facilitates Pol recruitment to stalled replication forks. *Molecular Biology of the Cell* **23**: 1943–1954.

Bienko M, Bienko M, Green CM, Crosetto N, Rudolf F, Zapart G, Coull B, Kannouche P, Wider G, Peter M, *et al.* 2005. Ubiquitin-Binding Domains in Y-Family Polymerases Regulate Translesion Synthesis. *Science* **310**: 1821–1825.

Bohgaki M, Bohgaki T, El Ghamrasni S, Srikumar T, Maire G, Panier S, Fradet-Turcotte A, Stewart GS, Raught B, Hakem A, *et al.* 2013. RNF168 ubiquitylates 53BP1 and controls its response to DNA double-strand breaks. *Proceedings of the National Academy of Sciences* **110**: 20982–20987.

Branzei D. **2011**. Ubiquitin family modifications and template switching. *FEBS Letters* **585**: 2810–2817. (review)

Buoninfante OA, Pilzecker B, Aslam MA, Zavrakidis I, van der Wiel R, van de Ven M, Berk PCM van den, Jacobs H, Buoninfante OA, Pilzecker B, *et al.* 2018. Precision cancer therapy: profiting from tumor specific defects in the DNA damage tolerance system. *Oncotarget* **9**: 18832–18843.

Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. **2005**. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes and Development* **19**: 1040–1052.

Ceccaldi R, Rondinelli B, D'Andrea AD. **2016**. Repair Pathway Choices and Consequences at the Double-Strand Break. *Trends in Cell Biology* **26**: 52–64. (review)

Ciccia A, Elledge SJ. **2010**. The DNA Damage Response: Making It Safe to Play with Knives. *Molecular Cell* **40**: 179–204. (review)

Daley JM, Sung P. **2014**. 53BP1 , BRCA1 , and the Choice between Recombination and End Joining at DNA Double-Strand Breaks. **34**: 1380–1388. (review)

Davies AA, Huttner D, Daigaku Y, Chen S, Ulrich HD. **2008**. Activation of Ubiquitin-Dependent DNA Damage Bypass Is Mediated by Replication Protein A. *Molecular Cell* **29**: 625–636.

Davies AA, Neiss A, Ulrich HD. **2010**. Ubiquitylation of the 9-1-1 checkpoint clamp is independent of Rad6-Rad18 and DNA damage. *Cell* **141**: 1080–1087.

Day TA, Palle K, Barkley LR, Kakusho N, Zou Y, Tateishi S, Verreault A, Masai H, Vaziri C. **2010**. Phosphorylated Rad18 directs DNA polymerase η to sites of stalled replication. *Journal of Cell Biology* **191**: 953–966.

Deshaies RJ, Joazeiro CAP. **2009**. RING Domain E3 Ubiquitin Ligases. *Annual Review of Biochemistry* **78**: 399–434.

Doré AS, Kilkenny ML, Rzechorzek NJ, Pearl LH. **2009**. Crystal Structure of the Rad9-Rad1-Hus1 DNA Damage Checkpoint Complex-Implications for Clamp Loading and Regulation. *Molecular Cell* **34**: 735–745.

Durando M, Tateishi S, Vaziri C. **2013**. A non-catalytic role of DNA polymerase η in recruiting Rad18 and promoting PCNA monoubiquitination at stalled replication forks. *Nucleic Acids Research* **41**: 3079–3093.

Falck J, Coates J, Jackson SP. **2005**. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* **434**: 605–611.

Gao Y, Mutter-Rottmayer E, Greenwalt AM, Goldfarb D, Yan F, Yang Y, Martinez-Chacin RC, Pearce KH, Tateishi S, Major MB, *et al.* 2016. A neomorphic cancer cell-specific role of MAGE-A4 in trans-lesion synthesis. *Nature Communications* **7**: 1–14.

Gervai JZ, Gálicza J, Szeltner Z, Zámborszky J, Szüts D. **2017**. A genetic study based on PCNA-ubiquitin fusions reveals no requirement for PCNA polyubiquitylation in DNA damage tolerance. *DNA Repair* **54**: 46–54.

Goodman MF, Woodgate R. **2013**. Translesion DNA polymerases. *Cold Spring Harbor Perspectives in Biology* **5**: 1–20.

Han J, Liu T, Huen MSY, Hu L, Chen Z, Huang J. **2014**. SIVA1 directs the E3 ubiquitin ligase RAD18 for PCNA monoubiquitination. *Journal of Cell Biology* **205**: 811–827.

Hanahan D, Weinberg RA. **2011**. Hallmarks of cancer: The next generation. *Cell* **144**: 646–674.

Hedglin M, Aitha M, Pedley A, Benkovic SJ. **2019**. Replication protein A dynamically regulates monoubiquitination of proliferating cell nuclear antigen. *Journal of Biological Chemistry* **294**: 5157–5168.

Helchowski CM, Skow LF, Roberts KH, Chute CL, Canman CE. **2013**. A small ubiquitin binding domain inhibits ubiquitin-dependent protein recruitment to DNA repair foci. *Cell Cycle* **12**: 3749–3758.

Hibbert RG, Huang A, Boelens R, Sixma TK. **2011**. E3 ligase Rad18 promotes monoubiquitination rather than ubiquitin chain formation by E2 enzyme Rad6. *Proceedings of the National Academy of Sciences* **108**: 5590–5595.

Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. **2002**. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* **419**: 135–141.

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

Hu Q, Botuyan MV, Cui G, Zhao D, Mer G. **2001**. Mechanisms of Ubiquitin-Nucleosome Recognition and Regulation of 53BP1 Chromatin Recruitment by RNF168-169 and RAD18. *Molecular Cell* **21**: 217.

Huang A, Hibbert RG, De Jong RN, Das D, Sixma TK, Boelens R. **2011**. Symmetry and asymmetry of the RING-RING dimer of Rad18. *Journal of Molecular Biology* **410**: 424–435.

Huang J, Huen MSY, Kim H, Leung CCY, Glover JNM, Yu X, Chen J. **2009**. RAD18 transmits DNA damage signalling to elicit homologous recombination repair. *Nature Cell Biology* **11**: 592–603.

Huertas P, Jackason SP. **2009**. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. *Journal of Biological Chemistry* **284**: 9558–9565.

Huttner D, Ulrich HD. **2008**. Cooperation of replication protein A with the ubiquitin ligase Rad18 in DNA damage bypass. *Cell Cycle* **7**: 3629–3633.

Inagaki A, van Cappellen WA, van der Laan R, Houtsmuller AB, Hoeijmakers JHJ, Grootegoed JA, Baarends WM. **2009**. Dynamic localization of human RAD18 during the cell cycle and a functional connection with DNA double-strand break repair. *DNA Repair* **8**: 190–201.

Inagaki A, Sleddens-Linkels E, van Cappellen WA, Hibbert RG, Sixma TK, Hoeijmakers JHJ, Grootegoed JA, Baarends WM. **2011**. Human RAD18 interacts with ubiquitylated chromatin components and facilitates RAD9 recruitment to DNA double strand breaks. *PLoS ONE* **6**: 1–14.

Jones J, Weber S, Prakash L. **1988**. The Saccharomyces cerevisiae RAD18 gene encodes a protein that contains potential zinc rimger domains for nucleic acid binding and a putative nucleotide binding sequence. *Nucleic Acids Research* **16**: 7119–7131.

Kannouche PL, Wing J, Lehmann AR. **2004**. Interaction of human DNA polymerase η with monoubiquitinated PCNA: A possible mechanism for the polymerase switch in response to DNA damage. *Molecular Cell* **14**: 491–500.

Keller J, Liu C, Ma T, Yu X, Wu J, Wang D. **2013**. RNF168 forms a functional complex with RAD6 during the DNA damage response. *Journal of Cell Science* **126**: 2042–2051.

Krijger PHL, Lee KY, Wit N, van den Berk PCM, Wu X, Roest HP, Maas A, Ding H, Hoeijmakers JHJ, Myung K, *et al.* **2011**. HLTF and SHPRH are not essential for PCNA polyubiquitination, survival and somatic hypermutation: Existence of an alternative E3 ligase. *DNA Repair* **10**: 438–444.

Leung W, Baxley RM, Moldovan GL, Bielinsky AK. **2019**. Mechanisms of DNA damage tolerance: post-translational regulation of PCNA. *Genes* **10**: 1–25.

Lin JR, Zeman MK, Chen JY, Yee MC, Cimprich KA. **2011**. SHPRH and HLTF Act in a Damage-Specific Manner to Coordinate Different Forms of Postreplication Repair and Prevent Mutagenesis. *Molecular Cell* **42**: 237–249.

Liu T, Chen H, Kim H, Huen MSY, Chen J, Huang J. **2012**. RAD18-BRCTx interaction is required for efficient repair of UV-induced DNA damage. *DNA Repair* **11**: 131–138.

Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, Lukas J. **2007**. RNF8 Ubiquitylates Histones at DNA Double-Strand Breaks and Promotes Assembly of Repair Proteins. *Cell* **131**: 887–900.

Mailand N, Gibbs-Seymour I, Bekker-Jensen S. **2013**. Regulation of PCNA-protein interactions for genome stability. *Nature Reviews Molecular Cell Biology* **14**: 269–282.

Masuda Y, Suzuki M, Kawai H, Suzuki F, Kamiya K. **2012**. Asymmetric nature of two subunits of RAD18, a RING-type ubiquitin ligase E3, in the human RAD6A-RAD18 ternary complex. *Nucleic Acids Research* **40**: 1065–1076.

Masutani C, Araki M, Yamada A, Kusumoto R, Nogimori T, Maekawa T, Iwai S, Hanaoka F. **1999**. Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. *EMBO Journal* **18**: 3491–3501.

Masuyama S, Tateishi S, Yomogida K, Nishimune Y, Suzuki K, Sakuraba Y, Inoue H, Ogawa M, Yamaizumi M. **2005**. Regulated expression and dynamic changes in subnuclear localization of mammalian Rad18 under normal and genotoxic conditions. *Genes to Cells* **10**: 753–762.

Matsuoka S, Huang M, Elledge SJ. **1998**. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* **282**: 1893–1897.

McManus TP, Woodgate R, Maher VM, McCormick JJ, Mead S, Wang Y. **2007**. Evidence that in Xeroderma Pigmentosum Variant Cells, which Lack DNA Polymerase η, DNA Polymerase ι Causes the Very High Frequency and Unique Spectrum of UV-Induced Mutations. *Cancer Research* **67**: 3018–3026.

Miyase S, Tateishi S, Watanabe K, Tomita K, Suzuki K, Inoue H, Yamaizumi M. **2005**. Differential regulation of Rad18 through Rad6-dependent mono- and polyubiquitination. *Journal of Biological Chemistry* **280**: 515–524.

Mutter-Rottmayer E, Gao Y, Vaziri C. **2016**. Cancer cells activate damage-tolerant and error-prone DNA synthesis. *Molecular & Cellular Oncology* **3**: e1225547. (review)

Nakajima S, Lan L, Kanno SI, Usami N, Kobayashi K, Mori M, Shiomi T, Yasui A. **2006**. Replication-dependent and -independent Responses of RAD18 to DNA damage in human cells. *Journal of Biological Chemistry* **281**: 34687–34695.

Ng BL, Kanaar R, Gergely F V., Morris BJ, Arends MJ, Bradley A, Tannahill D, Adams DJ, van der Weyden L, Markus A. **2005**. BRCTx Is a Novel, Highly Conserved RAD18-Interacting Protein. *Molecular and Cellular Biology* **25**: 779–788.

Niimi A, Brown S, Scott A, Sabbioneda S, Kannouche PL, Yasui A, Green CM, Lehmann AR. **2008**. Regulation of proliferating cell nuclear antigen ubiquitination in mammalian cells. *Proceedings of the National Academy of Sciences* **105**: 16125–16130.

Notenboom V, Hibbert RG, van Rossum-Fikkert SE, Olsen J V., Mann M, Sixma TK. **2007**. Functional characterization of Rad18 domains for Rad6, ubiquitin, DNA binding and PCNA modification. *Nucleic Acids Research* **35**: 5819–5830.

Palle K, Vaziri C. **2011**. Rad18 E3 ubiquitin ligase activity mediates fanconi anemia pathway activation and cell survival following DNA topoisomerase 1 inhibition. *Cell Cycle* **10**: 1625–1638.

Panier S, Ichijima Y, Fradet-Turcotte A, Leung CCY, Kaustov L, Arrowsmith CH, Durocher D. **2012**. Tandem Protein Interaction Modules Organize the Ubiquitin-Dependent Response to DNA Double-Strand Breaks. *Molecular Cell* **47**: 383–395.

Parrilla-Castellar ER, Arlander SJH, Karnitz L. **2004**. Dial 9–1–1 for DNA damage: the Rad9–Hus1–Rad1 (9–1–1) clamp complex. *DNA Repair* **3**: 1009–1014. (review)

Peng C-Y, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. **1997**. Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science* **277**: 1501–1505.

Potts PR, Porteus MH, Yu H. **2006**. Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks. *EMBO Journal* **25**: 3377–3388.

Poulsen M, Lukas C, Lukas J, Bekker-Jensen S, Mailand N. **2012**. Human RNF169 is a negative regulator of the ubiquitin-dependent response to DNA double-strand breaks. *Journal of Cell Biology* **197**: 189–199.

Prakash S, Johnson RE, Prakash L. **2005**. Eukaryotic Translesion Synthesis DNA Polymerases: Specificity of Structure and Function. *Annual Review of Biochemistry* **74**: 317–353.

Prakash S, Sung P, Prakash L. **1993**. DNA repair Genes and Proteins of Saccharomyces Cerevisiae. *Annual Review of Genetics* **27**: 33–70. (review)

Räschle M, Smeenk G, Hansen RK, Temu T, Oka Y, Hein MY, Nagaraj N, Long DT, Walter JC, Hofmann K, *et al.* **2015**. Proteomics reveals dynamic assembly of Repair complexes during bypass of DNA cross-links. *Science* **348**: 1-8.

Ravanat JL, Douki T, Cadet J. **2001**. Direct and indirect effects of UV radiation on DNA and its components. *Journal of Photochemistry and Photobiology B: Biology* **63**: 88–102. (review)

Rodrigue A, Lafrance M, Gauthier MC, McDonald D, Hendzel M, West SC, Jasin M, Masson JY. **2006**. Interplay between human DNA repair proteins at a unique double-strand break in vivo. *EMBO Journal* **25**: 222–231.

Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. **1998**. Double-stranded Brekas Induce Histone H2AX phosphorylation on Serine 139. *The Journal of Biological Chemistry* **273**: 5858–5868.

Rogozin IB, Goncearenco A, Lada AG, De S, Yurchenko V, Nudelman G, Panchenko AR, Cooper DN, Pavlov YI. **2018**. DNA polymerase η mutational signatures are found in a variety of different types of cancer. *Cell Cycle* **17**: 348–355.

Saberi A, Hochegger H, Szuts D, Lan L, Yasui A, Sale JE, Taniguchi Y, Murakawa Y, Zeng W, Yokomori K, *et al.* **2007**. RAD18 and Poly(ADP-Ribose) Polymerase Independently Suppress the Access of Nonhomologous End Joining to Double-Strand Breaks and Facilitate Homologous Recombination-Mediated Repair. *Molecular and Cellular Biology* **27**: 2562–2571.

San Filippo J, Sung P, Klein H. **2008**. Mechanism of Eukaryotic Homologous Recombination. *Annual Review of Biochemistry* **77**: 229–257. (review)

Sanchez Y, Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-worms H, Elledge SJ. **2008**. Conservation of the Chk1 Checkpoint Pathway in Mammals : Linkage of DNA Damage to Cdk Regulation Through Cdc25. *Science* **1497**: 1497–1502.

Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP. **2007**. Human CtIP promotes DNA end resection. *Nature* **450**: 509–514.

Sasatani M, Xu Y, Kawai H, Cao L, Tateishi S, Shimura T, Li J, Iizuka D, Noda A, Hamasaki K, *et al.* **2015**. RAD18 activates the G2/M checkpoint through DNA damage signaling to maintain genome integrity after ionizing radiation exposure. *PLoS ONE* **10**: 1–16.

Schwertman P, Bekker-Jensen S, Mailand N. **2016**. Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nature Reviews Molecular Cell Biology* **17**: 379–394. (review)

Sirbu BM, Cortez D. **2013**. DNA damage response: Three levels of DNA repair regulation. *Cold Spring Harbor Perspectives in Biology* **5**: 1–16. (review)

Sung P, Lamb J, Prakash S, Prakash L, Bailly V. **1994**. Specific complex formation between yeast RAD6 and RAD18 proteins: a potential mechanism for targeting RAD6 ubiquitin-conjugating activity to DNA damage sites. *Genes & Development* **8**: 811–820.

Takata M, Takeda S, Tachiiri S, Schild D, Sonoda E, Sasaki MS, Fukushima T, Thompson LH. **2002**. Chromosome Instability and Defective Recombinational Repair in Knockout Mutants of the Five Rad51 Paralogs. *Molecular and Cellular Biology* **21**: 2858–2866.

Tateishi S, Sakuraba Y, Masuyama S, Inoue H, Yamaizumi M. **2000**. Dysfunction of human Rad18 results in defective postreplication repair and hypersensitivity to multiple mutagens. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 7927–7932.

Thach TT, Lee N, Shin D, Han S, Kim G, Kim H, Lee S. **2015**. Molecular determinants of polyubiquitin recognition by continuous ubiquitin-binding domains of Rad18. *Biochemistry* **54**: 2136–2148.

Ting L, Jun H, Junjie C. **2010**. RAD18 lives a double life: Its implication in DNA double-strand break repair. *DNA Repair* **9**: 1241–1248. (review)

Trincao J, Johnson RE, Escalante CR, Prakash S, Prakash L, Aggarwal AK. **2001**. Structure of the Catalytic Core of S. cerevisiae DNA polymerase η: Implications for translesion DNA synthesis. *Molecular Cell* **8**: 417–426.

Ulrich HD, Jentsch S. **2000**. Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. *The EMBO Journal* **19**: 3388–3397.

Unk I, Hajdu I, Fatyol K, Hurwitz J, Yoon J-H, Prakash L, Prakash S, Haracska L. **2008**. Human HLTF functions as a ubiquitin ligase for proliferating cell nuclear antigen polyubiquitination. *Proceedings of the National Academy of Sciences* **105**: 3768–3773.

Unk I, Hajdu I, Fátyol K, Szakal B, Blastyak A, Bermudez V, Hurwitz J, Prakash L, Prakash S, Haracska L. **2006**. Human SHPRH is a ubiquitin ligase for Mms2-Ubc13-dependent polyubiquitylation of proliferating cell nuclear antigen. *Proceedings of the National Academy of Sciences* **103**: 18107–18112.

Verkade HM, Bugg SJ, Lindsay HD, Carr AM, O'Connell MJ. **2013**. Rad18 Is Required for DNA Repair and Checkpoint Responses in Fission Yeast. *Molecular Biology of the Cell* **10**: 2905–2918.

Vujanovic M, Krietsch J, Raso MC, Terraneo N, Zellweger R, Schmid JA, Taglialatela A, Huang JW, Holland CL, Zwicky K, *et al.* 2017. Replication Fork Slowing and Reversal upon DNA Damage Require PCNA Polyubiquitination and ZRANB3 DNA Translocase Activity. *Molecular Cell* **67**: 882-890

Walker JR, Corpina RA, Goldberg J. **2001**. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* **412**: 607–614.

Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, Zhang Y. **2004**. Role of histone H2A ubiquitination in Polycomb silencing. *Nature* **431**: 873–878.

Washington MT, Johnson RE, Prakash S, Prakash L. **1999**. Fidelity and processivity of Saccharomyces cerevisiae DNA polymerase η. *Journal of Biological Chemistry* **274**: 36835–36838.

Watanabe K, Iwabuchi K, Sun J, Tsuji Y, Tani T, Tokunaga K, Date T, Hashimoto M, Yamaizumi M, Tateishi S. **2009**. RAD18 promotes DNA double-strand break repair during G1 phase through chromatin retention of 53BP1. *Nucleic Acids Research* **37**: 2176–2193.

Watanabe K, Tateishi S, Kawasuji M, Tsurimoto T, Inoue H, Yamaizumi M. **2004**. Rad18 guides polη to replication stalling sites through physical interaction and PCNA monoubiquitination. *EMBO Journal* **23**: 3886–3896.

Weston R, Peeters H, Ahel D. **2012**. ZRANB3 is a structure-specific ATP-dependent endonuclease involved in replication stress response. *Genes and Development* **26**: 1558–1572.

Williams SA, Longerich S, Sung P, Vaziri C, Kupfer GM. **2011**. The E3 ubiquitin ligase RAD18 regulates ubiquitylation and chromatin loading of FANCD2 and FANCI. *Blood* **117**: 5078–5087.

Williams RS, Williams JS, Tainer JA. **2007**. Mre11–Rad50–Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochemistry and Cell Biology* **85**: 509–520.

Wit N, Krijger PHL, van den Berk PCM, Jacobs H. **2011**. Lysine residue 185 of Rad1 is a Topological but not a functional counterpart of lysine residue 164 of PCNA. *PLoS ONE* **6**: 1–7.

Xie C, Wang H, Cheng H, Li J, Wang Z, Yue W. **2014**. RAD18 mediates resistance to ionizing radiation in human glioma cells. *Biochemical and Biophysical Research Communications* **445**: 263–268.

Xin H, Lin W, Sumanasekera W, Yanbin Z, Wu X, Wang Z. **2000**. The human RAD18 gene product interacts with HHR6A and HHR6B. *Nucleic Acids Research* **28**: 2847–2854.

Yamazoe M, Szuts D, Simpson LJ, Kabani S, Sale JE. **2006**. Role for RAD18 in Homologous Recombination in DT40 Cells. *Molecular and Cellular Biology* **26**: 8032–8041.

Yanagihara H, Kobayashi J, Tateishi S, Kato A, Matsuura S, Tauchi H, Yamada K, Takezawa J, Sugasawa K, Masutani C, *et al.* **2011**. NBS1 Recruits RAD18 via a RAD6-like Domain and Regulates Pol η-Dependent Translesion DNA Synthesis. *Molecular Cell* **43**: 788–797.

Yang Y, Gao Y, Mutter-Rottmayer L, Zlatanou A, Durando M, Ding W, Wyatt D, Ramsden D, Tanoue Y, Tateishi S, *et al.* **2017**. DNA repair factor RAD18 and DNA polymerase Polκ confer tolerance of oncogenic DNA replication stress. *Journal of Cell Biology* **216**: 3097–3115.

Yeung M, Xiao W, Fu Y, Durocher D, Zhu Y, Zhang K. **2008**. Rad6-Rad18 Mediates a Eukaryotic SOS Response by Ubiquitinating the 9-1-1 Checkpoint Clamp. *Cell* **133**: 601–611.

Zeman MK, Cimprich KA. **2014**. Causes and consequences of replication stress. *Nature Cell Biology* **16**: 2–9. (review)

Zeman MK, Lin JR, Freire R, Cimprich KA. **2014**. DNA damage-specific deubiquitination regulates Rad18 functions to suppress mutagenesis. *Journal of Cell Biology* **206**: 183–197.

Zlatanou A, Sabbioneda S, Miller ES, Greenwalt A, Aggathanggelou A, Maurice MM, Lehmann AR, Stankovic T, Reverdy C, Colland F, *et al.* **2016**. USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. *Oncogene* **35**: 965–976.