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Review

DNA double-strand break repair: Putting zinc fingers on the sore spot

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ABSTRACT

Zinc-Finger (ZnF) proteins represent one of the most abundant group of proteins in the human genome. At first characterized as DNA binding proteins, it has become increasingly clear that ZnF-proteins have the ability to bind a large variety of substrates such as RNAs, proteins and post-translational modifications, suggesting potential roles in a variety of biological processes. Indeed, several studies have implicated ZnF-proteins for instance in transcription regulation, signal transduction and cell migration. Intriguingly, more recently these proteins have emerged as important protectors of the genome, particularly by orchestrating the repair of highly deleterious DNA double-strand breaks. Here we provide a comprehensive summary of the roles of ZnF domain-containing proteins in DNA double-strand break repair and discuss how their dysfunction impacts genome stability and human disease.

1. Introduction

Our genome is constantly challenged by endogenous and exogenous DNA damage, causing tens of thousands of DNA lesions on a daily basis [1]. DNA double-strand breaks (DSB) are considered one of the most toxic lesions that can occur in the genome. If left unrepaired or repaired inaccurately, they can lead to mutations and chromosomal translocations, thereby increasing the risk of developing human disorders such as cancer, neurodegeneration or immunodeficiency [2]. To protect the integrity of our genome, cells have evolved specialized molecular machines to detect and repair DSBs, the latter of which involves two main pathways: non-homologous end-joining (NHEJ) and homologous recombination (HR) [3]. Classical non-homologous end joining (cNHEJ) is the dominant pathway for DSB repair. During this repair process, the broken ends are bound by Ku70/Ku80, followed by the assembly of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and aprataxin and polynucleotide kinase/phosphatase-like factor (APLF). This leads to the PAXX-stimulated recruitment of the ligation machinery consisting of XRCC4, LIG4 and XLF, which ultimately seals the broken ends. cNHEJ requires no or minimal end-processing, the latter of which may lead to small deletions and insertions at the repair site [4]. HR, on the contrary, is the more faithful repair pathway and is restricted to the S and G2 phases of the cell cycle as it requires the presence of a sister chromatid. During HR, extensive resection of the DSB occurs involving the activities of endo- and exonucleases, including MRE11, CtIP, DNA2 and EXO1, to generate 3′single-strand (ss)DNA overhangs that become coated by the single-strand DNA binding complex RPA. This triggers the recruitment of BRCA1-PALB2-BRCA2 complexes, whose docking onto damaged DNA occurs in a manner dependent on physical interactions between PALB2 and RNF168 on the one hand, and RNF168 and ubiquitylated H2A on the other hand [5]. These events allow for the removal of RPA by BRCA2 and loading of the recombinase RAD51, which promotes homology search and strand invasion using the undamaged sister chromatid as a repair template [6]. When cNHEJ and HR are compromised, repair can occur via alternative non-homologous end joining (aNHEJ), which is an error-prone pathway that uses short stretches of microhomology to seal the broken ends. This pathway is dependent on XRCC1-Ligase III complex or the DNA polymerase POLQ [7]. Alternatively, larger stretches of resection can lead to repair by single-strand annealing (SSA), which is dependent on RAD52 and ERCC1 [8].

DNA repair pathway choice is strictly regulated throughout the cell cycle by the RING-finger E3 ubiquitin ligases RNF8 and RNF168, which promote the ubiquitin-dependent assembly of 53BP1 and the BRCA1-Abraxas-RAP80-MERIT40 (BRCA1-A) complex at DSBs [9]. 53BP1 inhibits DNA end-resection in G1 phase by its various effectors, including RIF1 and the Shieldin complex, to impair HR and favor NHEJ [10], whereas the BRCA1-A complex suppresses HR by sequestering BRCA1 away from the repair site. In addition, BRCA1 function, and consequently HR, are further restrained in G1 phase due to reduced BRCA1 expression in certain cellular contexts [11], as well as via inhibition of the interaction with PALB2, the latter of which involves the suppressive ubiquitylation of the BRCA1-interacting domain in PALB2 [12].

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Conversely, RIF1 accumulation at DSB sites in S and G2 phase is compromised by BRCA1 and CtIP, allowing end resection and HR to occur [13].

The repair of DSBs is challenged by the fact that genomic DNA is interweaved by histone and non-histone proteins in a high-order structure called chromatin. DNA repair machineries have to overcome this barrier to gain access to the damaged DNA and repair the lesions [14]. Over the recent years it became evident that chromatin structure is altered by different mechanisms such as DNA methylation, ATP-dependent chromatin remodeling and post-translational modifications (PTMs), including but not limited to phosphorylation, acetylation, methylation, S-acylation, poly(ADP)ribosylation (PARylation), SUMOylation and ubiquitylation [15,16]. A key Zinc-Finger (ZnF) protein that helps to overcome the chromatin barrier is PARP1. PARP1 is among the first proteins that binds to DNA breaks, where it promotes the rapid local expansion of chromatin by the formation of PAR-chains on itself and several target proteins, including histones [17]. This allows for the accumulation of DNA repair proteins, either by binding to DNA damage-associated PAR-chains or to the exposed damaged DNA, as was observed for several chromatin remodeling enzymes and cNHEJ factors, respectively [18,19]. In addition to PARP1, PARP3 also partakes in cNHEJ. This involves its auto-mono-ADP ribosylation (MARylation), which is a prerequisite for the interaction with APLF and the subsequent assembly of functional cNHEJ complexes during DSB repair [20].

Several studies reported that a number of transcription factors are also recruited to sites of DNA damage in a manner often dependent on PARP/PAR [18,21]. A large class of these transcription factors belongs to the ZnF domain-containing protein family, which represent one of the most abundant groups of proteins in the eukaryotic cell. It has become evident that ZnF protein function is not limited to transcription regulation, but is also key to many other cellular processes such as for instance signal transduction, cell migration and DSB repair [22]. Here

Table 1
Overview of ZnF proteins involved in DSB repair.

Domain name	Number of proteins	Number of proteins in DSB repair	Binding specificities	Examples	References
A20	7	1	Ubiquitin	A20/TNFAIP3[2−*]	[56]
ADD	4	1	DNA, modified histones	ATRX[2−*]	[65]
AN-1	8	0	DNA, RNA, Protein, Lipid		
B-Box	75	5	DNA	PML[2]	[82]
BED	6	0	DNA		
BTB/POZ	139	6	DNA, Protein	ZBTB7A[1], YY1[1], BACH1[2]	[83,84,85]
C2C2	6	1	DNA	TCEA[3]	[21]
C2H2	759	13	DNA, RNA, Protein, Lipid, Methylated DNA	ZBTB24[1−*], ZNF281[1−*], PHF11[2·*], ZNF830[2]	[32,33,53,98]
C2HC	6	3	DNA	RNF138[2−*]	[86]
C2CH	13	0	DNA		
C3H1	59	2	RNA	ZC3H11A[3]	[21]
C4	56	2	DNA	ESR2[3], NR1H4[3]	[21]
C5HC2	24	3	Modified histones	KDM4D[2]	[87]
CXXC	12	2	DNA	KDM2A[1−*]	[47]
CCHC	38	0	DNA, RNA		
CCHHC	7	0	DNA		
CHHC	4	0	RNA		
CW	7	1	Modified histones	MORC2[3]	[88]
DBF	3	0	DNA, Protein		
ePHD	23	1	Modified histones	PHF11[2−*]	[53]
FCS	5	2	RNA	L3MBTL2[1]	[89]
FYVE	32	0	Lipid, methylated DNA		
GATA	15	3	DNA	MTA2[3]	[21]
HIT	6	0	Protein, DNA		
KRAB	362	1	DNA, Ubiquitin	ZNF829[3]	[21]
LIM	71	0	Protein		
MATRIN	8	1	RNA	ZMAT1[3]	[21]
MIZ (SP-RING)	7	3	SUMO	PIAS1 and PIAS4[3]	[90]
MYM	6	1	SUMO	ZMYM3[2−*]	[66]
MYND	21	2	Protein	ZMYND8[2−*], SMYD3[2]	[91,92]
PARP	2	2	DNA	PARP1[3]	[19]
PBZ	2	2	Poly(ADP)ribose	APLF[1−*], CHFR[3]	[29,93]
PHD	71	27	Modified histones	PHF6[1−*], ACF1[1−*], KDM2A[2·*], KDM5A[2·*]	[36,39,46,80]
RAD18 (UBZ4)	8	3	Ubiquitin	RAD18[2−*]	[63]
RBZ (RANBP2)	23	4	Ubiquitin	RYBP[2−*]	[57]
RING	282	28	Protein, Ubiquitin	RNF8[1−*], RNF126[1−*], BARD1[2·*], RNF138[2·*], FRUCC[2·*]	[43,44,69,76,86]
SCA7	6	0	Protein		
SWIM	9	1	DNA, Protein	ZSWIM7[2]	[94]
TAZ	2	2	Protein, DNA	CBP/p300[1]	[95]
TFIIB	3	0	DNA		
TFIIS	6	1	DNA, RNA, Protein	TCEA1[3]	[21]
TRAF	23	0	Ubiquitin, Protein		
UBP	14	4	Ubiquitin	USP44[3], USP5[2]	[96,97]
ZBR	2	0	Protein		
ZZ	18	3	Protein	CBP/p300[1]	[95]

ZnF domains, the number of proteins containg a particular ZnF domain, the number of proteins implicated in DSB repair and their known binding affinities are indicated. Examples of ZnF proteins involved in DSB repair are shown, several of which play poorly understood roles in NHEJ (marked as [1]), HR (marked as [2]) or both NHEJ and HR (marked as [3]). Well-characterized ZnF domain-containing proteins in DSB repair, which are discussed in in this review, are marked with an asterisk (*).

we provide an overview of the current knowledge on the role of ZnF proteins in DSB repair, most notably NHEJ and HR, highlighting their importance as protectors of the genome.

2. Functional classification of ZnF domain-containing proteins

The ZnF domain was recognized over more than 30 years ago as a repeated zinc-binding motif consisting of conserved histidine and cysteine residues in the *Xenopus* transcription factor TFIIIA [23]. Over the years, numerous other zinc-binding domains have been identified, which are encoded by nearly 5 % of all human genes [24]. ZnF domains usually consist of multiple unique ZnF motifs that are responsible for the contact with different target molecules. The numerous structural folds, diverse binding modes and sequence recognition sites of ZnF motifs have led to the evolution of ZnF domain families. To date, ZnFs comprise more then 50 unique domains such as the FCS-, PBZ- and PHD-domains (Table 1). Although, ZnF motifs within a ZnF domain are evolved to bind zinc-ions, it has become clear that they are also capable of binding other metals in a cellular environment. For instance cobalt, cadmium, nickel and copper can compete with zinc for binding to ZnF domains. These metal-substitutions cause heteregenous structural and chemical changes of the ZnF motifs and thereby change their substrate-recognition properties [25].

While ZnFs are mostly known as DNA-binding domains, it has become clear that ZnF-domains also have the ability to bind RNA, lipids, and methylated DNA, as well as proteins and PTMs such as SUMO, ubiquitin, PAR and methyl (Fig. 1) [26]. Interestingly, many ZnF proteins possess multiple different types of ZnF domains. Consequently, they exhibit very different binding specificities for target molecules [22]. ZnF domains therefore also occur in several unrelated protein super-families (e.g. transcription factors, nuclear hormone receptors, integrase enzymes, E3 ubiquitin ligases, chromatin remodelers, tumor suppressors, RAS-GTPases, membrane transport proteins and chaperones) varying in domain structure and sequence, and displaying considerable versatility in their binding modes (some bind to DNA, others to RNA or proteins). This suggests that ZnF domains act as scaffolds that have evolved functions in a diversity of processes, including DSB repair (Fig. 1). In this review, we provide an overview of ZnF-proteins whose ZnF domains were shown to be functionally relevant for NHEJ or HR.

3. ZnF proteins implicated in NHEJ repair of DNA breaks

ZnF domain-containing proteins have been implicated in the repair of DSBs via NHEJ. Below we discuss the function of several of these ZnF domain-containing proteins in this repair process (Table 1).

3.1. PBZ-type ZnF protein: APLF

First classified as a classical C2H2 domain-containing protein, structural and biochemical studies revealed that APLF contains shorter inter-cysteine-histidine loops which have binding specificity for PAR. This led to the classification of APLF as a member of the PBZ-domain family [27]. Interestingly, the two PBZ-domains of APLF bind to auto-MARylated PARP3 at sites of DNA damage. APLF also contains a Ku-binding motif (KBM) that mediates its interaction with Ku and is important for APLF's recruitment to sites of DNA damage. Moreover, APLF interacts with XRCC4 via its N-terminal fork-head associated domain (Fig. 2A) [20]. This multitude of interactions mediated by APLF ensures the binding and retention of LIG4 through its cofactors XLF and XRCC4, thereby promoting cNHEJ [28]. Importantly, mutations in the PBZ-domains, as well as in the KBM motif, abolished APLF recruitment, impaired XRCC4 loading and compromised both the efficiency and accuracy of NHEJ, indicating the relevance of its ZnF domain for DSB repair [29].

3.2. C2H2-type ZnF proteins: ZBTB24 and ZNF281

Two classical C2H2-type ZnF proteins, ZBTB24 and ZNF281, have been implicated in NHEJ. Sequence analysis revealed that ZBTB24 contains a BTB- or POK- (POZ and Krüppel ZnF) domain, which is frequently found in transcription factors [30], as well as a Krüppel-type C2H2 ZnF domain. Interestingly, mutations in ZBTB24 are causally linked to immunodeficiency, centromeric instability and facial anomalies (ICF2) syndrome [31]. We unveiled that loss of ZBTB24 in both B cells from mice and ICF2 patients causes a cNHEJ defect during immunoglobulin class-switch recombination (CSR) and consequently impaired immunoglobulin production [32]. Domain-mapping revealed that the C2H2-, but not the BTB- domain of ZBTB24 has PAR-binding affinity and mediates its interaction with PARP1-associated PAR-chains, an event that is important for ZBTB24 recruitment to DNA breaks. Moreover, the C2H2-domain of ZBTB24 protects PAR-chains on PARP1 and this is critical for the assembly of XRCC4-LIG4 complexes and cNHEJ at DNA breaks (Fig. 2B) [32]. Together, these findings show the importantce of a C2H2-type ZnF protein in cNHEJ, providing a molecular basis for the observed CSR defect in ICF2 patients.

The C2H2 domain-containing protein ZNF281 was identified in a screen aimed to measure the localization of transcription factors at laser micro-irradiation induced DNA damage [21]. ZNF281 recruitment to sites of DNA damage was dependent on its C2H2-domain and the activity of PARP1 [33]. Whether its recruitment relies on binding of the C2H2-domain to DNA damage-associetd PAR-chains or the exposed damaged DNA remains to be established. ZNF281 also interacts with XRCC4, thereby promoting its loading at DNA breaks. Interestingly,

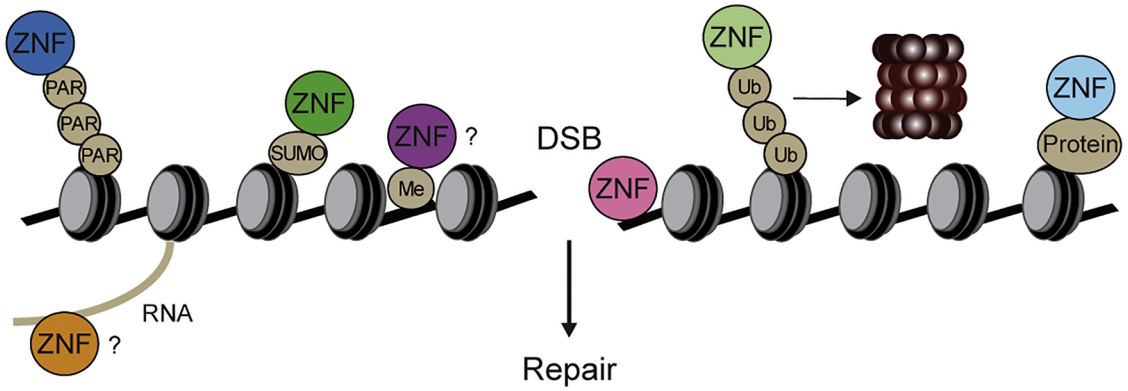

Fig. 1. Binding specificities of zinc-finger (ZnF) domains.
At DSBs, ZnF domains bind to a variety of substrates including DNA, proteins and PTMs such as PAR, SUMO and K48-linked ubiquitin chains, to regulate DSB repair. It remains to be established whether the binding of ZnF domains to RNA and methylated DNA has functional relevance for DSB repair (?).

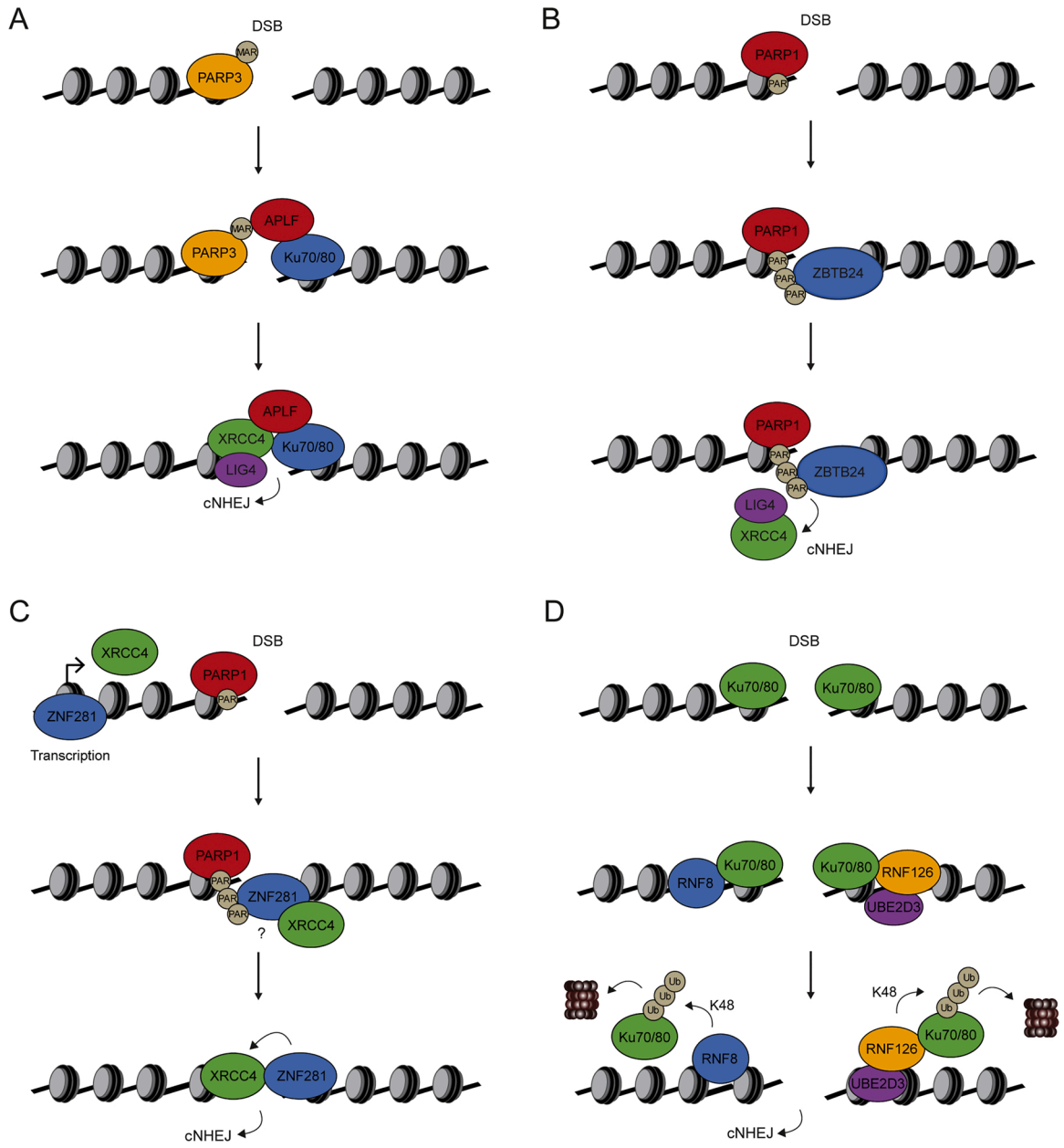

Fig. 2. Model for the roles of ZnF-proteins APLF, ZBTB24, ZNF281, RNF8 and RNF126 in cNHEJ.
(A) APLF is recruited to DSBs by binding to PARP3-associated MAR through its PBZ-domain, and by associating with Ku70/Ku80. It also interacts with XRCC4, thereby promoting recruitment of XRCC4/LIG4 and cNHEJ. **(B)** ZBTB24 is recruited to DSBs by binding to PARP1-associated PAR through its C2H2-domain, thereby functioning as a scaffold to protect PAR from degradation. This is followed by the PARP1-dependent recruitment of XRCC4/LIG4 and cNHEJ. **(C)** ZNF281 is recruited to DSBs via PARP activity and through its C2H2-domain. Whether the latter involves its binding to PAR or DNA is unclear. The C2H2-domain also interacts with XRCC4, thereby facilitating XRCC4 recruitment and cNHEJ. ZNF281 also binds to the *XRCC4* promotor via its C2H2-domain, thereby controlling XRCC4 expression and cNHEJ. **(D)** RNF8 and RNF126 are recruited to DSBs, where they modify Ku70/Ku80 by K48-ubiquitylation, triggering the proteasome-dependent degradation of Ku70/Ku80 during cNHEJ. RNF126 does so by interacting with Ku70/Ku80, an event that is also critical for its recruitment, and by operating with the E2 conjugating enzyme UBE2D3.

point-mutations in ZNF281′s C2H2-motifs abolished the interaction with XRCC4, impaired XRCC4 recruitment to sites of DNA damage and lead to a defect in cNHEJ (Fig. 2C) [33]. Interestingly, however, another study reported that ZNF281 binds to the promotor of the *XRCC4* gene in manner dependent on its C2H2-domain, thereby controlling *XRCC4* expression and cNHEJ (Fig. 2C) [34]. Future studies will have to clarify precisely how ZNF281′s apparent dual mode-of-action impacts this repair process.

3.3. PHD-type ZnF proteins: PHF6 and ACF1

PHD-domains were discovered more than 25 years ago and numerous sequence and functional analyses of PHD-containing proteins have pinpointed a role in the regulation of chromatin structure [35]. Their intimate association with histones and their ability to recruit multi-protein complexes to damaged chromatin has suggested important roles for these proteins in DNA-based processes, including DNA repair [35]. Indeed, two PHD-type ZnF proteins, PHF6 and ACF1, have been implicated in NHEJ.

PHF6 and several other PHD-domain family-members such as PHF3

and PHF12, were among the top hits in a G2-checkpoint recovery RNAi-screen [36]. PHF6 contains two PHD-domains of which PHD1 is responsible for its localization in the nucleolus, where it interacts with the ribosomal DNA transcription factor UBF [37], while PHD2 mediates its binding to dsDNA in vitro [38]. PHF6 is recruited to DSBs in a PAR/PARP-dependent manner, where it promotes 53BP1 accummulation and repair via NHEJ. Importantly, G2-checkpoint recovery and 53BP1 accumulation were impaired in cells expressing a mutant form of PHF6 lacking both its PHD-domains. Although the exact molecular mechanism underlying PHF6′s role in G2-checkpoint recovery remains to be established, the existing data suggest that the PHD-domains play a pivotal role in promoting 53BP1-dependent NHEJ, thereby preventing persistent unrepaired DSBs that inhibit recovery from a G2-checkpoint arrest [36].

ACF1 is a non-catalytic PHD-domain containing subunit of the ACF1-ISWI chromatin remodeling complex. ACF1 binds to the central part of core histones (H2A, H2B, H3 and H4) and is recruited to sites of DNA damage through its PHD-domain [39,40]. Moreover, ACF1 recruits components of the ACF1-ISWI complex, most notably its catalytic ATPase subunit SNF2H, as well as Ku70/Ku80 to DSBs. The latter is dependent on the interaction between ACF1 and Ku70/Ku80 and SNF2H-driven chromatin remodeling. In addition, ACF1 and SNF2H also promote DSB repair by HR in cooperation with the ACF1-associated proteins CHRAC15 and CHRAC17 in the ISWI-complex, which induce chromatin changes to target ACF1/SNF2H to damaged chromatin. This is followed by the SNF2H-dependent reruitment of HR-factors such as RPA and RAD51 at DSBs [41]. Taken together, these data suggest that chromatin changes induced by SNF2H are important for both HR and NHEJ. The latter is dependent on SNF2H-dependent recruitment of ACF1, which in turn leads to the loading of Ku70/Ku80 at DSBs, thereby facilitating repair of these lesions via NHEJ [39].

3.4. RING-type ZnF proteins: RNF8 and RNF126

RING-domains are present in E3 ubiquitin ligases and make direct contact with E2 ubiquitin conjugating enzymes to ensure ubiquitin transfer to target proteins [42]. Two RING finger domain-containing proteins have been implicated in NHEJ. RING-finger protein RNF8 is involved in the proteasomal degradation of Ku80 through its Lys48-linked ubiquitination. This event triggers the release of Ku80 from DNA damage sites. While it is evident that the RING-domain is required for the ubiquitination of Ku80, the E2 enzyme involved in this process remains to be identified (Fig. 2D) [43]. More recently, RNF126, in conjunction with the UBE2D3 E2 conjugating enzyme, was found to associate with Ku80 via its RING-domain. Similar to RNF8, RNF126 also ubiquitylates Ku80 to trigger its release from DNA damage sites during NHEJ [44]. Although, RNF8 and RNF126 both promote cNHEJ by regulating Ku70/Ku80 release during DSB repair, unclear is whether they cooperate or act redundantly during this process (Fig. 2D).

3.5. PARP-type ZnF protein: PARP1

The PARP-type ZnF protein family-member poly(ADP-ribosyl) polymerase 1 (PARP1) is a versatile protein involved in distinct DSB repair pathways including cNHEJ [19]. PARP1 becomes activated upon DNA damage, modifies itself and target proteins by the covalent addition of long branched polymers of ADP-ribose, which leads to the recruitment of chromatin remodelling enzymes and several cNHEJ repair factors [19,41]. PARP1 consists of an N-terminal DNA binding domain comprising two ZnF domains, ZnF1 and ZnF2. Mutational disruption of the ZnF domains confirmed their necessity for PARP1 recruitment and retention at sites of DNA damage in vivo [45]. Structural analysis revealed dimerization between ZnF1 and ZnF2 from two different PARP1 molecules, which specifically recognize the single strand-double strand transition of a DNA break [45]. Importantly, the DNA damage-dependent dimerization of the two ZnF domains provides the catalytic domain access to the PAR acceptor sites, thereby enabling PAR signaling. These findings illustrate the importance of the ZnF domains in PARP1 for its recruitment, dimerization and activation, the latter of which is critical for PAR signaling and DSB repair.

4. ZnF proteins implicated in HR repair of DNA breaks

In addition to the role of ZnF domain-containing proteins in NHEJ, it became evident that ZnF proteins are also involved in DSB repair by HR. Below we discuss the function of several of these ZnF domain-containing proteins in this repair process (Table 1).

4.1. PHD and CXXC-type ZnF protein: KDM2A

PHD domain-containing proteins have not only been implicated in NHEJ (see above), but also impact HR. For instance, the PHD and CXXC domain-containing protein KDM2A interacts with and becomes phosphorylated by the DSB-responsive kinase ATM at threonine 632 located within its PHD-motif. This counteracts KDM2A's binding to damaged chromatin and enhances H3K36me2 levels at DSB sites. Moreover, H3K26me2 serves as a platform to recruit MRE11 via its binding partner NBS1, which binds this histone mark via its BRCT2 domain. Utlimately, this faciliates HR by promoting MRE11-dependent end resection (Fig. 3A) [46]. Interestingly, however, a more recent study reported a role for the CXXC-domain in the recruitment of KDM2A to *bona fide* nuclease-induced DSBs [47]. In addition, proteomics approaches identified 53BP1 as a binding partner of KDM2A. The KDM2A-53BP1 interaction was found to be dependent on the CXXC-domain of KDM2A and was required for the KDM2A-mediated ubiquitination and recruitment of 53BP1. Unclear is how this histone demethylase promotes 53BP1 ubiquitylation and how this modification of 53BP1 affects its accumulation at DNA damage sites (Fig. 3A). Nevertheless, KDM2A-depleted cells displayed impaired 53BP1 foci formation, elevated levels of unrepaired DSBs and premature exit from the G2/M checkpoint. Re-expression of a ΔCXXC-version of KDM2A failed to rescue these defects and resulted in an increase of micronuclei formation [47]. These findings suggest that the ZnF domains of KDM2A support DSB-repair through distinct modes. While its impact on HR is evident, it remains unclear whether KDM2A also affects NHEJ. A function in this latter process may perhaps be expected given its role in regulating 53BP1. Future studies will therefore have to resolve precisely how KDM2A dictates DSB-repair outcome.

4.2. C2H2-type ZnF proteins: CTCF

CTCF is a multifunctional protein commonly known for its role in genome organziation and transcription [48]. CTCF contains 11 ZnF motifs comprising ten C2H2 motifs and one C3H1 motif. Mutations in either of these motifs impair DNA binding and nuclear mobility [49]. Interestingly, proteomics approaches identified CTCF as a DNA damage-dependent binding-partner of MRE11 and CtIP. These interactions were found to be dependent on its ZnF domain and facilitate CtIP recruitment to DNA breaks, allowing end-resection and HR to take place [50]. In line with this, CTCF was also described to interact with, and recruit RAD51 and BRCA2 at sites of DNA damage [51,52], suggesting a multifaceted role during different stages of the HR process.

4.3. ePHD-type ZnF protein: PHF11

PHF11, which is a member of the extended PHD (ePHD) family of ZnF proteins, contains a ZnF domain that consists of two parts. One of which is the pre-PHD region that binds a single zinc ion, and the other is a PHD-finger motif that binds two additional zinc-ions. PHF11 was identified at uncapped telomeres using the proteomics of isolated chromatin segments (PICh) approach, and its PHD-finger motif was found to interact with RPA, suggesting it may act at resected DNA ends

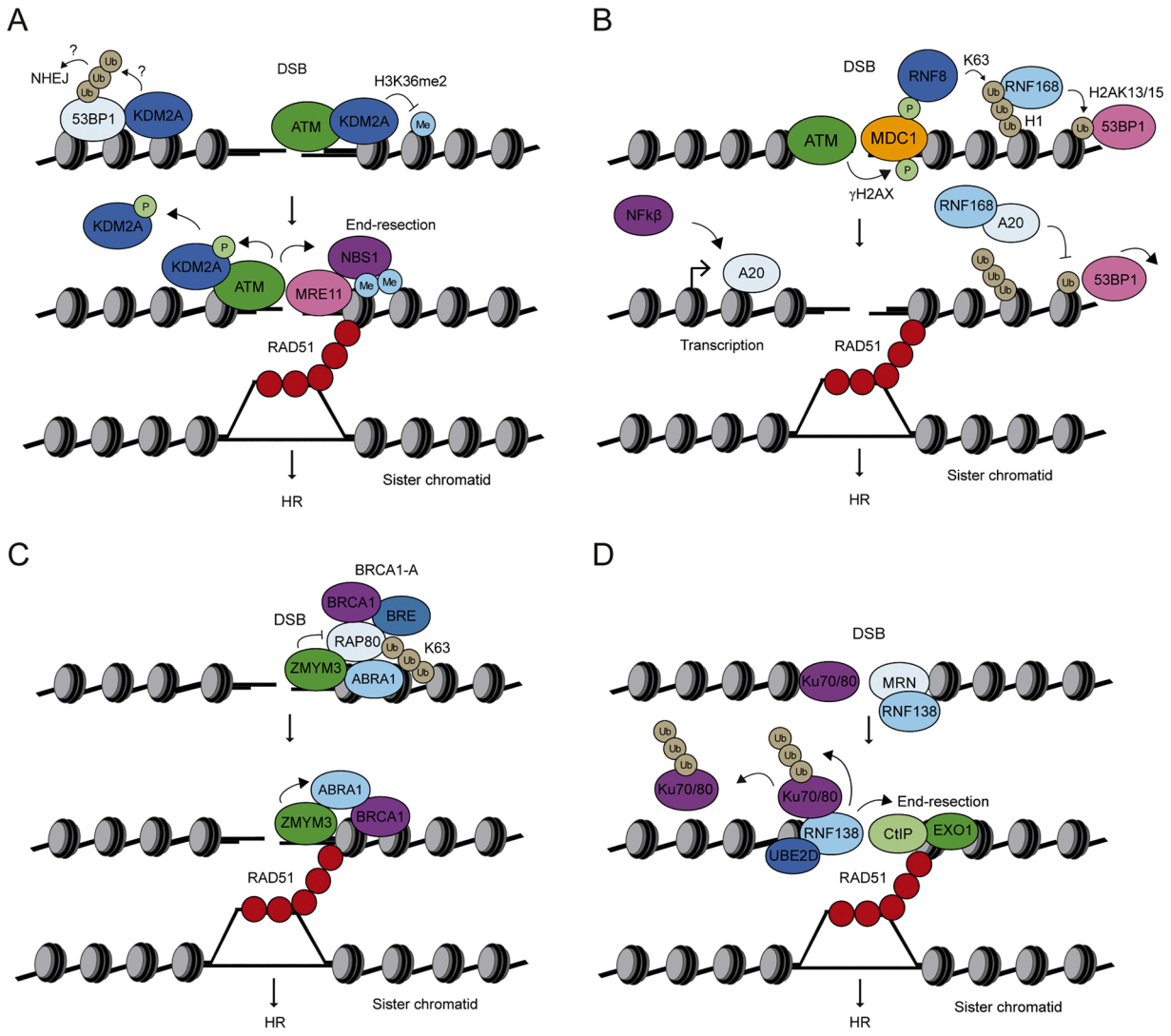

Fig. 3. Model for the roles of KDM2A, A20/TNFAIP3, ZMYM3 and RNF138 in HR.
(A) ATM-induced phosphorylation of KDM2A's PHD-domain counteracts its chromatin-binding, resulting in increased H3K36me2 levels at DSBs. This enhances the binding of MRE11 via its interaction partner NBS1, which binds to H3K36me2, thereby stimulating end-resection and HR. KDM2A also interacts with 53BP1 through its CXXC-domain promoting the ubiquitination-dependent recruitment of 53BP1 via an unknown mechanism. Whether KDM2A promotes 53BP1-dependent NHEJ is equally unclear. **(B)** In response to DSBs, A20 is transcriptionally upregulated by NFkβ. A20 binds to RNF168 through its ZnF domain, abrogating RNF168-binding to ubiquitinated H1. This impairs the RNF168-dependent ubiquitination of H2AK13/15 and accrual of 53BP1, allowing end-resection and HR to occur. **(C)** ZMYM3 is recruited to DSBs through interactions with H2A/H2AX and dsDNA, and via its interaction partners in the BRCA1-A complex (RAP80, BRE and ABRA1). ZMYM3 facilitates the recruitment of ABRA1 and BRCA1 to DSBs, while antagonizing the HR-suppressive effects of BRCA1-A, thereby facilitating RAD51 loading and HR. **(D)** Ku70/80 and MRN complexes bind DSBs independently. In S/G2 phase, MRE11 processes DSB ends to create short overhangs, which are recognized and bound by the ZnF domains of RNF138. This leads to the displacement of DNA-bound Ku70/80 complex through RNF138-UBE2D-mediated ubiquitylation. Ku removal allows for binding of the CtIP/EXO1 nucleases, which further resect the DSB ends, thereby promoting HR.

[53]. Indeed, PHF11 mediates the removal of RPA, thereby providing access for EXO1 or DNA2 to partially resected ends generated by MRN, which are otherwise inaccessible for these nucleases [53]. Consequently, loss of PHF11 impaired HR and rendered cells sensitive to DSB-inducing agents. Whether PHF11 is recruited to DNA breaks via its PHD domain-dependent interaction with RPA or via its ePHD-domain remains to be established.

4.4. A20-type ZnF protein: A20/TNFAIP3

The DSB-response involves the RING-type ZnF proteins and E3 ubiquitin ligases RNF8 and RNF168. RNF8 interacts with ATM-phosphorylated MDC1 and ubiquitylates histone H1 to recruit RNF168 via its motifs interacting with ubiquitin (MIU). RNF168 then catalyzes the mono-ubiquitination of H2A and H2AX, which initiates the subsequent formation of K63-linked ubiquitin chains for the assembly of the BRCA1-A complex and 53BP1, that latter of which promotes NHEJ [9]. A recently discovered zinc-finger protein A20/TNFAIP3 was described to function in the RNF168-53BP1 axis. This protein contains an A20-type ZnF domain that was identified in a cDNA-based screen for regulatory factors in the tumor necrosis factor (TNF) signaling cascade. Sequence analysis showed that this domain contains multiple repeats of Cys_2/Cys_2 fingers [54]. Structural analysis and functional assays confirmed that A20 binds mono-ubiquitin and K63-linked poly--ubiquitin chains [55]. Indeed, following its transcriptional upregulation by NFkβ after DNA damage, A20 directly binds to RNF168 via its ZnF domain. This disrupts the binding of RNF168 to ubiquitinated H2A and H1, thereby antagonizing RNF168-dependent ubiquitylation and 53BP1 binding at DNA damage sites (Fig. 3B) [56]. Accordingly, loss of A20 lead to increased NHEJ levels, concomitantly with a decrease in HR. This

establishes a new link between NFkβ-signaling and the regulation of an A20-type ZnF protein during DSB repair pathway choice.

4.5. RanBP2-type ZnF protein: RYBP

RYBP belongs to the non-canonical PcG protein complex and possesses a ubiquitin-binding motif (UBM) within its RanBP2-domain, which may be involved in the recognition and/or amplification of ubiquitin at DSBs. Indeed, RYBP preferentially binds to K63-linked ubiquitin chains via its ZnF domain to suppresses BRCA1 binding. However, upon DNA damage RYBP's ZnF domain becomes poly-ubiquitinated by RNF8, which leads to its rapid removal from damaged chromatin by the VCP/p97 segregase, allowing BRCA1 recruitment and repair via HR to occur [57]. This implies a dual function of the RanBP2-type motif, which on one hand binds to ubiquitin, and on the other hand is required for the ubiquitination-dependent removal of RYBP. A similar behavior was described for the ZnF proteins TAB2 and TAB3, which belong to the same family [58], suggesting a widespread role for RanBP2-type ZnF proteins in HR.

4.6. UBZ4-type ZnF protein: RAD18

The ability of cells to repair DSBs via HR relies on the recombinase RAD51. Vertebrates contain five different RAD51 paralogs which form two distinct protein complexes. Mutations in any of these paralogs leads to defects in HR and genome instability [59]. One such paralog, RAD51C, is regulated by the UBZ4-type ZnF protein RAD18. Similar to the more classical C2H2 ZnFs, such as those found in the UBZ and UBM domains of Y-family polymerases [60], the UBZ4-type ZnF was also shown to bind to ubiquitin [61]. Indeed, RAD18 binds to K63-linked ubiquitin chains generated by RNF8/UBC13 through its UBZ4-domain, where it associates with RAD51C via its RING-domain, serving as an adaptor for RAD51C loading on damaged chromatin [62,63]. Importantly, both UBZ4- and RING-domain mutants failed to rescue the HR-defect observed in RAD18-depleted cells, illustrating the importance of these ZnF domains in regulating RAD18-dependent HR. Although it is evident that RAD18 affects HR by promoting RAD51C loading at DSBs, precisely how RAD51C impacts this repair process remains unclear.

4.7. ADD-type ZnF protein: ATRX

The ADD-domain of ATRX consist of an N-terminal GATA-like ZnF domain and a PHD-finger. However, the PHD-finger of ATRX is different from the classical PHD-finger domains found in PHF2 and KDM2A, as it consists of an additional N-terminal C2C2 motif. Sequence analysis revealed that the only proteins that share this feature are DNMT3 and DNMT3L. Hence, the domain is called ATRX-DNMT3-DNMT3L (ADD) [64]. ATRX recruitment to sites of DNA damage and its binding to damaged chromatin rely on the imperfect PHD domain [65]. These events are followed by H3.3 incorporation, which occurs in conjunction with the ATRX chaperone DAXX, and is required for extended repair synthesis following RAD51-dependent strand invasion during HR.

4.8. MYM-type ZnF protein: ZMYM3

The MYM-domains are only found in six mammalian proteins, one of which was identified in a comparative proteomic analysis as a chromatin-interacting protein [66]. This protein, ZMYM3, was also found to interact with members of the BRCA1-A complex (RAP80, ABRA1 and BRE) and to promote BRCA1-A accumulation at sites of DNA damage, particularly by regulating ABRA1 accrual. While the BRCA1-A complex is known to inhibit HR by restricting end-resection in the S/G2 phase of the cell cycle, ZMYM3 was found to counteract the RAP80-dependent accumulation of BRCA1-A at DNA damage sites, allowing BRCA1-PALB2-BRCA2-RAD51-mediated HR to occur (Fig. 3C) [67]. ZMYM3 binds DNA and chromatin via its N-terminal domain, which is distinct from its MYM domain. Importantly, both the N-terminus and the MYM domain of ZMYM3 are required for its recruitment to sites of DNA damage [66]. The exact binding substrate of ZMYM3′s MYM domain remains, however, to be determined.

4.9. RING-type ZnF proteins: BARD1, RNF138 and FRUCC

BARD1 is a RING-type ZnF protein whose RING-finger is required for its dimerization with BRCA1. This stimulates BARD1-BRCA1 E3 ubiquitin ligase activity and the ubiquitylation of H2A at sites of DNA damage [68]. Ubiquitylated H2A serves as a binding platform for the ATP-dependent chromatin remodeler SMARCAD1, which repositions or evicts nucleosomes, thereby counteracting 53BP1-mediated inhibition of DNA resection [69]. On the other hand, BARD1-BRCA1 also bind to DNA, specifically to the D-loop formed after RAD51-dependent strand invasion, a process that is enhanced by BRCA1-BARD1 through direct interaction with RAD51. Thus, both the RING-finger dependent E3 ubiquitin ligase activity and the DNA-binding capabilities of BRCA1 and BARD1 contribute to efficient HR repair [70].

MRE11-RAD50-NBS1 have been implicated in the removal of Ku70/Ku80 from DSBs, allowing end-resection and HR to occur [71]. Another protein that supports HR by the removal of Ku is the E3 ubiquitin ligase RNF138, which, besides a RING domain, also contains a C2HC and C2H2 domain. While the deletion of either of these domains separately did not affect RNF138 recruitment to DSBs, deleting all domains simultaneously completely abolished its recruitment, particularly its binding to ssDNA overhangs at these DNA lesions. Consequently, it remains unclear which combination of domains is responsible for its DNA binding. Nevertheless, it is evident that the RING domain mediates RNF138′s interaction with Ku70/Ku80. RNF138, in conjunction with the E2 UBED2, ubiquitylates Ku70/Ku80 in a manner dependent on its RING, C2HC and C2H2 domains to promote Ku70/Ku80 eviction from DSBs. This in turn allows for the recruitment of CtIP/EXO1 and extensive end-resection, promoting DSB repair by HR (Fig. 3D) [72]. Importantly, RNF138 was also found to interact with the RAD51 paralog RAD51D and promote the ubiquitin-dependent proteasomal degradation of RAD51D via its RING domain. Moreover, RNF138 depletion resulted in decreased RAD51 foci formation and increased levels of chromosomal aberrations. However, precisely how RAD51D impacts HR remains unclear [73,74].

Since DSBs occur in both inactive and actively transcribed regions, it is of utmost importance that transcription and DNA repair are coordinated properly. This is to prevent collisions between transcription and DNA repair machineries that may otherwise interfere with DSB repair. Indeed, several studies have observed a direct link between transcription repression and HR [75], and implicated a role for the FRUCC-complex in regulating these processes [76]. FRUCC was identified as an E3 ubiquitin ligase complex consisting of FBXL10 and the RING-domain proteins RNF68-RNF2, which ensure the recruitment of the BMI-RNF2 and MEL18-RNF2 E3 ubiquitin ligase complexes. These complexes are responsible for H2A-K119 ubiquitylation, a mark associated with the repression of transcription. In addition, FRUCC also promotes the exchange of H2A with H2A.Z and thereby represses transcription [76]. Loss of FRUCC results in a defect of transcriptional silencing and impaired the loading of HR proteins at DSBs, thereby affecting this repair process.

4.10. MYND-type ZnF protein: ZMYND8

The MYND-domain consists of a ZnF-motif that primarily mediates protein-protein interactions during transcription regulation [77]. One of the MYND-domain containing proteins, ZMYND8, was identified as a factor in a screen for bromo-domain proteins that localize at sites of DNA damage [78]. ZMYND8 contains a triple PHD-BRD-PWWP chromatin-binding module in its N-terminus and a C-terminal MYND domain. The PHD-BRD-PWWP domain is responsible for binding acetylated histones and DNA. Proteomics analysis revealed that ZMYND8 associates

with the NuRD chromatin remodeling complex, as well as with the ZnF proteins ZNF532, ZNF592 and ZNF687, all of which are recruited to sites of DNA damage. The MYND domain in ZMYND8 binds to a PPPLΦ motif in the NuRD subunit and GATA-type ZnF protein GATAD2A. This interaction is important for the rapid, PAR/PARP-dependent recruitment of GATAD2A/NuRD to sites of DNA damage [79]. In addition, the association of ZMYND8-NuRD with damaged chromatin also depends on the removal of H3K4me3, which is a mark associated with active transcription. The H3K4me3 demethylase and PHD containing ZnF protein KDM5A, which is recruited to sites of damage via PAR and its PHD-domain, was shown to ensure ZMYND8-NuRD binding by demethylation of H3K4me3 [80]. Taken together, these studies demonstrated that the combined action of several ZnF proteins ensures H4K4me3 demethylation at DSBs, allowing ZMYND8-NuRD to bind the damaged chromatin, repress transcription and promote HR [78].

5. Conclusions and future perspectives

ZnF domains are present in in at least 5 % of all human proteins [24]. Their numerous structural folds and sequence recognition motifs allow them to bind to a plethora of substrates. The fact that ZnF proteins often contain multiple different ZnF domains and that the sequence recognition motifs within a domain exhibit different binding-specificities, also allows them to recognize a combination of substrates. Due to these multifaceted features, ZnF proteins have been implicated in a broad range of molecular processes. Over the recent years, considerable efforts have also highlighted important roles of ZnF proteins in DSB repair (Table 1). Several studies have demonstrated a role for ZnF domains in facilitating the recruitment and binding of DNA repair factors to damaged chromatin by regulating PTMs, chromatin remodeling, protein-protein interactions and/or transcription. Besides the somewhat more well-described role of some larger ZnF domain families in DSB repair (PHD and RING), the functional relevance and mode-of-action of the majority of these proteins in this process remains poorly understood. Not only is it unclear to which substrates and/or combinations of substrates different ZnF domain-containing proteins bind to, also the lack of biochemical and cellular complementation studies with mutant proteins lacking functional ZnF domains disallowed their characterization during DSB repair.

Interestingly, several uncharacterized ZnF proteins have been shown to localize at sites of laser micro-irradiation induced DNA damage in a manner often dependent on PAR/PARP [21]. Laser micro-irradiation is a widely used method to characterize the spatiotemporal dynamics of proteins using imaging of fluorescent proteins at DNA damage sites in living cells or fixed cells. This method induces a wide range of DNA lesions, including DSBs, single-strand breaks and oxidative DNA lesions, suggesting that ZnF proteins may have the ability to bind different types of DNA lesions. Moreover, ZnF proteins also ranked high in CRISPR/-Cas9 screens aimed at identifying protein networks that protect cells against DNA damaging agents, including chemotherapeutics [81]. Collectively, this work suggests a broader role of ZnF proteins in DNA repair and tumor resistance mechanisms than previously anticipated.

Emerging evidence has shown that several ZnF proteins also play key roles in the development of human diseases such as cancer and neurodegeneration [22]. For instance, ZNF281 is overexpressed in colorectal cancer (CRC) and causes cancer metastasis through regulation of epithelial to mesenchymal transition (EMT) [22]. Mutations in ZBTB24 are causally linked to ICF syndrome [31], whereas ZMYM3 mutations are frequently present in several cancers, including chronic lymphocytic leukemia (CLL), medulloblastoma, and Ewing sarcoma [24]. It is, however, not entirely understood how mutations and changes in the expression of ZnF proteins contribute to disease etiology, warranting the further functional characterization ZnF proteins in human diseases associated with DNA repair alterations. Moreover, mutational signature analyses through next generation sequencing will likely expand our knowledge on ZnF-mutations and their link to human disease, most notably cancer. Such knowledge may not only lead to a better understanding of disease mechanisms, but may also pave the way for the development of drugs that target ZnF proteins in anti-cancer therapies.

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Declaration of Competing Interest

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Appendix A. Supplementary data

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