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Caught with one's zinc fingers in the genome integrity cookie jar

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Abstract

Zinc finger (ZnFs) domains exist in at least 5% of all human proteins. First characterized to bind DNA, ZnFs display extraordinary binding plasticity including RNA, lipids, proteins and post-translational modifications. The diverse binding properties of ZnFs have made their functional characterization challenging. While once confined to large and poorly characterized protein families — proteomic, cellular and molecular studies have begun to shed light on their involvement as protectors of the genome. Here we focus on the emergent roles of ZnF domain-containing proteins in promoting genome integrity, including their involvement in telomere maintenance and DNA repair. These findings have highlighted the need for further characterization of ZnF proteins, which can reveal functions of this large gene class in normal cell function and human diseases, including those involving genome instability such as aging and cancer.

Keywords

DNA damage; DNA double-strand breaks; DNA repair; genome integrity; telomeres; zinc finger

Protection of Genome Integrity

Genetic information is stored within the base sequences of DNA and its integrity is preserved by a sophisticated DNA damage machinery, termed the **DNA damage response (DDR)** (see Glossary), which has evolved to detect and repair DNA lesions [1, 2]. The most deleterious type of DNA lesions are **DNA double-strand breaks (DSBs)** that, if left unrepaired, can result in loss of entire portions of chromosomes and its associated genomic information. Moreover, DSBs, when repaired incorrectly result in genome instability, including mutations, chromosomal translocations and chromosome mis-segregation [3]. DSBs occur in response to endogenous and exogenous DNA damaging agents, including reactive oxygen species, replication stress, aberrant nuclease activity and genotoxic cancer

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therapies [1, 2, 4]. A particular class of DSBs are the natural ends of chromosomes, the **telomeres**, that if left unprotected are recognized as DSBs by the cellular DNA damage response machinery [5, 6]. Cells have evolved several mechanisms to deal with DSBs. At telomeres, specific telomere-binding proteins cap the chromosome termini and hide them so that these structures are not recognized as sites of DNA damage [7]. However, when telomeres become critically short, these protective proteins are lost resulting in the activation of the DDR at chromosome ends. Similarly, other chromosomal DNA lesions resulting in DSBs activate the DDR [1, 2, 4, 8]. This large network of proteins involved in the DDR then signals and activates cell cycle checkpoints and DNA repair activities to allow repair of the lesion and coordination with the cell cycle machinery to ensure that DNA replication and cell division do not occur in the presence of unrepaired DNA damage. These pathways are vital for the maintenance of genome integrity as telomere dysfunction or defects in the DDR contribute to human diseases including immunological disorders, neurodegeneration, aging and cancer [1, 2, 4, 6, 9, 10].

DNA-templated reactions including transcription and replication, as well as repair of DNA, rely on proteins that bind to DNA to locally regulate these processes. Eukaryotic DNA is bound by histone proteins and organized into **chromatin**, which regulates DNA accessibility and function, including genome integrity pathways [11, 12]. In addition to interacting with DNA, histone proteins are also highly modified by **post-translational modifications (PTMs)**, including phosphorylation, acetylation, methylation, ubiquitylation and sumoylation, which regulate chromatin structure and function [13, 14]. This regulation includes PTMs functioning as recognition signals, which are bound by proteins resulting in their recruitment and accumulation onto chromatin [15–18]. For the DDR, it is clear that local concentration of proteins on DNA and/or chromatin is vital to compartmentalize these reactions in space and time so as to allow these proteins to exert their functions in a coordinated manner [19]. This spatio-temporal relationship of factors is regulated in part by nucleic acid binding as well as PTM recognition and protein-protein interactions at genomic loci.

In human cells, the largest class of DNA binding proteins are zinc finger domain-containing proteins, which are encoded by nearly 5% of all human genes [20]. **Zinc finger (ZnFs) domains** often contain cysteine and histidine residues that coordinate the binding of zinc. ZnFs can be broadly characterized into numerous unique domains (Interpro and SMART databases, Table 1). Zinc fingers were first described as a DNA binding domain in the *Xenopus laevis* transcription factor IIIA [21]. The ability to bind DNA in a sequence-specific manner made ZnFs synonymous with site-specific DNA binding transcription factors [22]. However, ZnFs also bind RNA and other diverse macromolecules. For example, ZnFs mediate protein-protein interactions and bind to other modified proteins including PTMs such as ubiquitin, SUMO, methylated histones and poly(ADP-ribose) (Figure 1, Key Figure) [23–25]. The discovery of broad binding specificities of zinc fingers has reshaped our thinking of the function of these domains within proteins and their versatility in promoting molecular interactions in cells. In addition to gene regulation, well-characterized zinc finger domains, such as RING and PHD, which mediate ubiquitin signaling and chromatin interactions respectively, play crucial roles in genome integrity. However, recent

work has identified other broad classes of ZnF proteins that promote telomere and genome integrity.

Numerous publications have recently begun to unravel the functions of previously uncharacterized ZnF proteins in telomere maintenance and the DDR. Here, we focus our review on newly characterized ZnF proteins that have only recently been identified as important players in these pathways. These examples suggest that many uncharacterized ZnF proteins are likely to have functions that extend beyond gene regulatory roles involving DNA binding. Based on these studies, we propose a need to re-evaluate and determine the function of ZnF proteins and their binding specificities more broadly, which could shed light on how these large classes of proteins orchestrate processes that safeguard the genome to ensure normal cell function and the suppression of human diseases.

Zinc Finger Proteins involved in the DNA Damage Response

Transcription factors

C2H2 zinc finger proteins represent one of the largest families of regulatory proteins in mammals. This class of proteins plays a role in a variety of cellular activities, such as development, cellular differentiation, genome integrity and tumor suppression. The C2H2 represents the classical zinc finger domain, and it plays a critical role in binding to DNA sequences with specific affinity provided by amino acids in the α -helix of the finger aided by the conserved linkers present between fingers. The human genome contains over 650 C2H2 zinc finger proteins (see Table 1). Transcription factors (TFs) make up a large proportion of these proteins (Table 1) [20, 22]. Recently, a large-scale DNA damage recruitment screen identified TFs, including ZnF proteins, as a large class of DNA damage response factors [26]. Using **laser microirradiation**, Elledge and colleagues screened 576 proteins for their ability to accumulate at laser damage and identified over 120 proteins as being DNA damage localized [26]. Interestingly, 34 of these proteins contained ZnF domains. Gene ontology analysis of the validated factors identified DNA binding and transcriptional functions as being over-represented in this list. Based on this analysis, an additional screen was performed on 35 randomly selected TFs that were not previously associated with the DDR and which came from diverse categories including ZnF proteins. Of these, 71% localized to DNA damage sites, including proteins that contain the ZnF domains C2H2, C4, and KRAB [26]. Mutation analysis revealed that several of these factors required DNA binding activities, including the ZnF domain of NR1H4, to support damage recruitment. The observed damage association were dependent on PARP activity as inhibitors targeting PARP1 and PARP2 blocked their damage recruitment.

Given these results, several hypotheses can be considered to understand the role transcription factors have in the DDR. This study, as well as others, has observed chromatin decompaction at sites of DNA damage [26, 27]. It is conceivable that DNA damage-associated relaxation of chromatin may unmask DNA sequences, allowing unprogrammed transcription factor binding. It is also possible that transcription factors localize to sites of DNA damage to regulate chromatin and transcription to promote DDR activities [28–30]. Regardless, future studies are warranted to test and determine the function of TFs in orchestrating DNA damage signaling and repair within chromatin. Given the prevalence of

TF binding to damage sites, it is tempting to speculate that there are DDR pathways that regulate this phenomenon to ensure that DNA and chromatin-associated proteins, including DDR factors and TFs, are coordinated in a spatio-temporal fashion at damage sites so that DNA repair can occur efficiently within this environment. In this context, association of TFs and transcriptional activity in proximity to sites of damage could interfere with the recruitment of DDR factors and the repair of DNA lesions. Given the prevalence of transcription dysregulation and DDR defects in cancer, understanding the relevance of these findings in both normal and diseased states is critical.

C2H2 zinc finger containing protein involved in genome stability: Aprataxin and ZATT

Several proteins containing C2H2 domains in addition to transcription factors play an important role in genome integrity and utilize the zinc finger domain to recognize important altered DNA structures. For example, Aprataxin, a gene mutated in the neurological disorder known as ataxia oculomotor apraxia, is involved in resolving abortive DNA ligation intermediates arising upon oxidative free radicals [31]. Aprataxin is an enzyme that catalyzes the release of cytotoxic 5'-adenylation DNA termini (5'-AMP), allowing DNA termini to serve again as substrates for DNA ligases. The C-terminal ZnF domain of Aprataxin provides high affinity binding to AMP-DNA target sites allowing efficient AMP-DNA hydrolase activity [32, 33]. Inactivating mutations affecting Aprataxin, including some mutations that result in truncations lacking the ZnF domain [34], result in the accumulation of DNA damage and ultimately death of cells with high levels of oxidative metabolism, such as neurons.

The Zinc finger protein Associated with TDP2 and TOP2 (ZATT, also known as ZNF451) is a SUMO E3 ligase and DNA repair factor that contains multiple C2H2-type zinc fingers [35–37]. ZATT is involved in processing stalled protein-DNA Topoisomerase 2 cleavage complexes (TOP2cc) [37]. TOP2 normally creates DNA double-strand breaks (DSBs), which form transient intermediates where DNA is bound to TOP2 (i.e. TOP2cc), which are used to resolve DNA topological stress, including DNA supercoiling that forms during transcription and replication [38–41]. Abortive TOP2 reactions can result in genome instability and several drugs used as frontline anticancer therapies, including **etoposide** and doxorubicin, poison the TOP2 reaction to trap the enzyme to DNA thereby creating the potentially cytotoxic TOP2cc protein-DNA adduct [4, 40, 42]. ZATT was very recently shown to aid in the resolution of stalled TOP2cc by stimulating sumoylation of TOP2cc, which is bound by TDP2 [37]. TDP2 activity is also stimulated by ZATT, which promotes the resolution of protein-DNA crosslinks involving TOP2 [37]. ZATT modulates the cellular response to etoposide as ZATT deficiency results in defects in repair and cell survival of etoposide-induced TOP2 damage. Given the importance of TOP2 poisons in treating cancer, ZATT function in cancers and scenarios where TOP2 inhibitor drug resistance occurs should be analyzed. In addition, mechanistically, it is unclear what role the zinc finger domains play in regulating ZATT function. Given the involvement of DNA and SUMO in ZATT activities with TOP2, it would be interesting to see whether or not the zinc finger domains of ZATT participate in DNA and/or SUMO binding that promote its function in resolving TOP2-DNA adducts.

MYM-type zinc finger: ZMYM3

The MYM-type zinc finger is found in 6 mammalian proteins. These proteins are characterized by a region of tandem zinc-binding MYM (myeloproliferative and mental retardation) motifs [43], whose name was derived after being identified in genes mutated in X-linked mental retardation [44] and a myeloproliferative disorder [45]. Recently, the MYM family member ZMYM3 (zinc finger [ZNF], myeloproliferative, and mental retardation-type 3) was shown to be involved in DSB repair [46].

A comparative proteomic analysis of histone H2A variant interacting proteins identified ZMYM3 as a chromatin recognition protein [46]. Further analysis revealed that ZMYM3 associates with DNA damage sites where it acts to promote repair of DSBs by **homologous recombination (HR)**. These findings along with the observation that deletion of ZMYM3 in human cancer cells results in genome instability and DNA damage sensitivity suggest that ZMYM3 is a DDR factor. In addition to chromatin, ZMYM3 also interacts with the **BRCA1-A complex**. The involvement of ZMYM3 in HR appears to stem from its ability to promote the accumulation of the HR factor BRCA1 at DNA damage sites and relieve the suppressive function of the RAP80-containing BRCA1-A complex to allow productive repair of DSBs to occur by HR [46, 47]. Collectively, ZMYM3 appears to modulate the association and interactions of the BRCA1-A complex with BRCA1, chromatin and DNA damage sites, to allow fine-tuning of these factors to regulate repair of DSBs by HR.

The MYM-type zinc fingers are important for the DDR functions of ZMYM3 as deletion of these motifs impair its interactions with damage sites and ability to facilitate effective repair. It is not known whether or not MYM-type zinc fingers bind DNA, although there are two proteins in this family that have been shown to bind the post-translational modification, SUMO through their MYM-type zinc fingers [48]. However, the biological significance of this binding is unknown. Interestingly, ZMYM3 interacts with DNA through a N-terminal DNA binding domain, which is not contained within the MYM zinc fingers [46]. In addition to binding DNA and histones, we speculate that ZMYM3 may also utilize the MYM-type zinc fingers to mediate additional interactions within chromatin. As SUMO has been shown to be an important PTM involved in DNA damage signaling and repair [49], it remains to be determined whether or not ZMYM3 utilizes SUMO binding to facilitate its DDR functions. In addition, other MYM-containing proteins have not been analyzed in the DDR.

The involvement of ZMYM3 in the DDR is one of the only functions reported for MYM-containing proteins. Interestingly, ZMYM3 is frequently mutated in several cancers including chronic lymphocytic leukemia (CLL), medulloblastoma, and Ewing sarcoma [50–53]. ZMYM3 has also been implicated as one of three top genes associated with progression of Alzheimer's disease [54]. Male infertility and defects in meiotic cell cycle progression were observed in mice lacking ZMYM3 [55]. Given these results and the involvement of ZMYM3 in DNA repair, additional studies are needed to understand how ZMYM3 is involved in human diseases. ZMYM3 KO cells display PARP inhibitor sensitivity, a current treatment that is being used and developed for treating HR-deficient cancers [46]. It is tempting to speculate that ZMYM3 mutations may contribute to cancer, and potentially

other diseases, as a consequence of altered DNA repair associated with ZMYM3 deficiencies [46].

MYND-type zinc finger: ZMYND8

The MYND domain is a member of the treble clef zinc finger domain family and is found in several proteins involved in chromatin and development. Named after 3 proteins that contain this domain (i.e. MYeloid translocation protein 8, Nery and DEAF-1), the MYND domain has been shown to promote protein-protein interactions, including the specific binding to PXLXP motifs, which was first demonstrated for the binding between the MYND domain of BS69 (also known as ZMYND11) and several viral and cellular proteins [56]. The MYND domain is also found in several proteins containing a SET domain, which make up the SMYND family of histone methyltransferases [57]. ZMYND8 and ZMYND11, two chromatin interacting proteins that contain the triple chromatin reader domain module - PHD, bromodomain and PWWP domain – also contain a MYND domain in their C-terminus. Recently, several studies identified ZMYND8 as an important chromatin **reader protein** involved in DSB repair and the DDR in human cells (Figure 2) [16, 28, 58–60].

ZMYND8 was first identified as a DNA damage factor in a screen for bromodomain proteins that relocate upon DNA damage in human cells [58]. Using laser microirradiation, ZMYND8 was shown to accumulate at DNA damage sites. Proteomic analysis revealed that ZMYND8 binds the NuRD chromatin remodeling complex and regulates its association with DNA damage sites. Interestingly, ZMYND8 binds the NuRD complex through its MYND domain, which binds a PPPLΦ motif in the NuRD associated factor GATAD2A [60]. Functionally, ZMYND8-NuRD, as well as the recently identified ZnF-containing histone demethylase KDM5A, facilitates transcriptional repression at damage sites (Figure 2) [28, 58, 61]. This pathway also promotes repair of DSBs through the HR pathway.

Numerous ZnF proteins regulate the KDM5A-ZMYND8-NuRD DDR axis (Figure 2). In addition to ZMYND8, this complex also interacts with additional ZnF proteins, ZNF532, ZNF592 and ZNF687 (i.e. the Z3 module), which associate with DNA damage regions and are also involved in regulating the ZMYND8-NuRD DDR axis [58, 60, 61]. Loss of ZNF687 results in defective damage recruitment of the NuRD factor CHD4 but neither ZNF687 nor ZNF592 are required for ZMYND8 accrual at damage sites, suggesting a complex involvement of the Z3 module in regulating interactions between NuRD, ZMYND8 and damaged chromatin [60, 61]. The functions of the ZnF domains within the Z3 module are unknown. In addition to the MYND domain, ZMYND8 also contains a ZnF domain within the triple chromatin reader module, which is vital for chromatin recognition and damage recruitment of ZMYND8 [59]. Both PARP1 and KDM5A contain ZnFs (Table 1) and each of these proteins is required for ZMYND8-NuRD recruitment to damage sites [60, 61]. The ZnF domains of PARP1 have been shown to recognize DNA to promote its dimerization, DNA damage recruitment and activation [62]. KDM5A contains 3 PHD domains, with PHD1 binding unmodified histone H3 and PHD3 binding its substrate, H3K4me3 [63]. Interestingly, the PHD1 domain of KDM5A is required for the accumulation of this factor with damage sites while PHD3 is dispensable. However, both PHD1 and PHD3 are required to promote ZMYND8-NuRD associations with DNA damage. These results are in line with

other data showing that the demethylation of H3K4me3 is vital to allow ZMYND8-NuRD to associate with and function within chromatin containing DNA lesions [61]. Taken together, the KDM5A-ZMYND8-NuRD DDR axis provides an example for the multiple roles that ZnFs perform in genome integrity pathways (Figure 2). In addition to DNA binding, protein and modified histone interactions mediated by these domains are required for this pathway. Given the number of ZnFs involved in this pathway, additional molecular, cellular and biochemical analyses are warranted to determine the molecular mechanisms that govern these interactions within chromatin to orchestrate the DDR functions of this pathway. It is likely that this pathway could provide a paradigm for understanding multivalent interactions involving ZnFs that mediate DNA repair and signaling within the chromatin environment.

Other zinc finger domains involved in the DDR

In addition to those described in detail previously, many other ZnF proteins have been implicated in genome integrity (see Table 1 and its associated references). For example, RING and PHD domain-containing proteins represent large classes of ZnF proteins involved in genome maintenance. Another important ZnF domain-containing protein is RAD18, whose 6 family members all have DNA damage functions. These proteins include the E3 Ub ligase RAD18 and Spartan, which modify and bind PCNA respectively to promote the DDR during replication stress [64, 65]. SNM1A contains a ZnF that binds RAD18-dependent ubiquitin substrates at damage sites to promote DNA repair [66]. ZRANB3, a DNA translocase, contains a RBZ ZnF domain that binds poly-ubiquitylated PCNA upon replication stress [67]. These PCNA ubiquitin pathways collectively ensure that replicative DNA damage is repaired to maintain genome integrity. The ZnF domain UBP is found in ubiquitin proteases that also mediate DDR functions. These include USP16 and USP51, which deubiquitylate histones to promote the DDR [30, 68].

There are several other examples of ZnF proteins that also utilize PTMs for their DDR functions. The aprataxin polynucleotide-kinase-like factor (APLF) contains two PAR-binding zinc finger (PBZ) domains that bind poly(ADP-ribose), a modification that is important in the DDR [69]. SUMO, a small ubiquitin-like protein, is added to proteins at DNA damage sites by SUMO E3 ligases, including PIAS4 and MMS21, which both contain MIZ ZnF domains [49, 70]. The histone acetyltransferases CBP and p300, which are involved in the DDR, contain TAZ and ZZ ZnF domains [71]. The ZnF protein ZNF668 interacts with the histone acetyltransferase TIP60 and is required for histone acetylation and chromatin relaxation that promote HR repair of DSBs [72]. Taken together, these studies highlight the critical involvement of ZnF proteins in DDR processes that rely on PTMs, which demonstrates that in addition to interacting with nucleic acids, ZnF domains also promote diverse macromolecular interactions that drive DNA damage responses including signaling and repair.

Zinc Finger Proteins involved in Telomere Maintenance: ZSCAN4, ZNF827 and TZAP

Recently, proteins containing C2H2 ZnFs have emerged as key regulators of telomere length in mammalian cells (Figure 3). In mammals, telomeres consist of long tracts of repetitive

DNA sequences (TTAGGG) bound by a specific protein complex termed shelterin [5]. Two opposing forces determine telomere length in mammalian cells, telomere erosion, and telomere lengthening mechanisms. Telomeres shorten during each cell cycle division due to the so-called “end replication problem” [73]. Telomerase counteracts telomere erosion by synthesizing new telomeric repeats using its reverse transcriptase activity. An alternative mechanism of telomere extensions, the so-called alternative lengthening of telomeres (ALT) pathway, engages the DDR pathway to extend telomeres using existing telomeric sequences as a template. The first zinc finger protein shown to play a critical role at mammalian telomeres is ZSCAN4 (zinc finger and SCAN domain-containing protein 4). ZSCAN4 is expressed and is required for telomere extension observed during the early stages of embryogenesis. Recent studies suggest that ZSCAN4 promotes genome-wide DNA demethylation that in turn increases telomerase-mediated telomere elongation as well as increased telomere recombination (Figure 2) [74]. Depletion of ZSCAN4 or mutation of the 4th ZnF-domain in its C-terminus results in loss of DNA demethylation and associated defects in telomere shortening and recombination, highlighting the relevance of this factor in telomere length regulation during a key stage of development.

Another zinc finger protein shown to play a role at telomeres is the C2H2 zinc finger protein ZNF827 [75]. This protein is enriched at telomeres in cells maintaining telomeres using the ALT pathway. Recruitment of ZNF827 at ALT telomeres is mediated by the nuclear hormone receptors, TR4 and COUP-TF2 which bind to variant telomeric repeats (TCAGGG), frequently found at telomeres in ALT cells [75]. When ZNF827 binds telomeres, it recruits the NuRD nucleosome-remodeling and histone-deacetylation complex leading to changes in chromatin structure. Localization of the NuRD–ZNF827 complex at telomeres facilitates the recruitment of DNA repair proteins to promote HR and telomere elongation mediated by the ALT pathway (Figure 2).

The zinc finger and BTB containing protein TZAP (aka ZBTB48) has been shown to bind directly to the canonical telomeric repeats (TTAGGG) [76, 77]. TZAP contains 11 C2H2 zinc finger domains, 3 of which bind specifically to TTAGGG repeats. Interestingly, TZAP localization to telomeres is inhibited by the shelterin complex and, as a result, it is found at telomeres with reduced shelterin levels. In particular, hyper-elongated telomeres have a low density of shelterin and abundant levels of TZAP. Under these conditions, TZAP promotes a rapid deletion of telomeric sequences through a mechanism known as “telomere trimming” (Figure 2) [78]. In this context, TZAP appears to play a crucial role to set an upper limit to telomere length, thereby preventing telomeres from becoming extremely elongated, a condition associated with genome instability [79]. Interestingly, in this setting competition for binding to TTAGGG repeats between the zinc fingers of TZAP and the Myb-domains of the shelterin components TRF1 and TRF2 provides a mechanism to establish telomere length homeostasis.

Concluding Remarks

The recent studies reviewed here highlight the significant contribution of ZnF proteins to genome integrity pathways. Given that a large number of ZnF proteins are uncharacterized or untested for their ability to influence these pathways (Table 1), additional studies are

needed to comprehensively define the function of ZnF proteins, including in genome maintenance pathways. As part of this endeavor (see Outstanding Questions), many challenges remain. ZnF domains are diverse and are encoded in many genes. The rules that govern the regulation and function of these proteins are still being determined. As ZnFs, even within the same domain class, can exhibit varied binding specificities, it is unclear how these interactions are regulated. Regardless, the studies presented here highlight the spectrum of macromolecular interactions by ZnF proteins that drive molecular interactions that orchestrate genome maintenance pathways, in addition to those afforded by sequence-specific DNA binding. High-throughput technologies including next-generation sequencing, CRISPR-Cas9 genome editing, proteomics and systems biology approaches could assist in the deconvolution of ZnF protein functions both in normal and diseased states. Focused large-scale studies of ZnF proteins have not been reported, making it difficult to assign functions to these proteins broadly. Genome maintenance pathways may be particularly reliant on ZnF proteins given that interactions between DNA, RNA, protein and PTMs play pivotal roles in the DDR and telomere maintenance. Understanding the molecular interactions governed by ZnF proteins and how these proteins function across diverse genomic and cellular environments is essential for understanding genome regulation and maintenance, including the DDR and telomeres. As genome dysfunction impacts not only cancer and aging but many human diseases, understanding ZnF functions and their biological processes are likely to inform both basic and translational research of the various and numerous ZnF human proteins.

Outstanding Questions

What are the functions and binding specificities of each unique zinc finger domain? Does each ZnF always bind the same substrate or can they be regulated or diversified by sequence variations, modifications, and/or protein interactions between the ZnF protein and protein interacting partners? How are these interactions controlled and integrated into DDR processes involved in genome integrity maintenance?

What are the genomic binding sites and cellular localization of zinc finger proteins? What are the protein-binding partners of zinc finger proteins?

How do multiple zinc finger proteins within a functional network regulate multivalent interactions with various substrates to build a complex to mediate its DDR function(s)?

Will recent advances in high-throughput proteomic, genomic and loss of function CRISPR/Cas9 techniques allow for the deconvolution of these large, diverse protein families into functional classes?

Could reconstitution of multi ZnF-containing protein complexes, including with DNA and chromatin, be analyzed by Cryo-EM to allow complex, multivalent interactions to be captured and analyzed?

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Glossary

BRCA1-A Complex

one of at least four complexes containing BRCA1 that is recruited to DNA double-strand breaks. In this complex, BRCA1 interacts with RAP80, BRCC36, BRCC45, MERIT 40, and adaptor protein Abraxas.

Chromatin

a complex of DNA and protein that constitutes the eukaryotic chromosome. Its basic unit is the nucleosome; made up of 146 bp of DNA wrapped around an octamer of histone proteins. It organizes and compacts DNA into the nucleus and regulates DNA accessibility and function.

DNA damage response (DDR)

a large network of proteins that sense, detect and repair damaged DNA to preserve genomic integrity.

DNA double-strand breaks (DSBs)

one of the most dangerous types of DNA damage that is a result from breakage of both strands of the DNA. DSBs can trigger cell death and genome instability involving chromosome translocations and loss, as well as other mutations.

Etoposide

a Topoisomerase 2 poison used as a chemotherapeutic drug to treat various cancers. It acts by trapping the Topoisomerase 2 protein onto DNA, which results in DNA double-strand breaks and cytotoxicity.

Homologous recombination (HR)

one of the primary pathways that repair DNA double-strand breaks. This pathway utilizes a homologous template to restore the damaged DNA molecule during S and G2 phases of the cell cycle. This process involves DNA end resection to create a 3' overhang that can invade the template followed by synthesis and resolution of the new DNA.

Laser microirradiation

a method that uses laser-induced DNA damage to study defined DNA damage sites in cells. This technique is widely used in DDR studies to characterize spatio-temporal dynamics of proteins using immunofluorescence or imaging of fluorescent proteins at DNA damage sites in fixed or living cells.

Post-translational modifications (PTMs)

the covalent modification of proteins that occur on the side chains of amino acids or at the protein termini. Common PTMs include phosphorylation, acetylation, methylation, SUMOylation and ubiquitylation. Histones are highly modified PTMs, which regulate chromatin dynamics and function.

Reader protein

proteins that contain specific domains that bind PTMs. Examples include chromo-, PHD-, BRCT- and bromo- domains. Bromodomains recognize and bind acetylated lysine residues, including on histones, which functions to promote interactions, localization and cellular functions of proteins in cells.

Telomere

a segment of DNA at the ends of chromosomes consisting of repetitive sequences, which serve to cap and protect the chromosome ends. Telomeres also solve the end-replication problem through the engagement of telomerase, an enzyme that maintains the length of telomeric DNA. Telomere dysfunction is associated with several human diseases including cancer and aging.

Zinc finger (ZnF) domain

a protein domain that coordinates one or more zinc ions to stabilize its finger-like structure. Numerous unique zinc finger domains exist. Initially characterized to bind to DNA, these domains can mediate diverse macromolecular interactions with additional substrates including RNA, lipids, proteins, and PTMs including ubiquitin, SUMO and Poly(ADP-ribose).

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Highlights

- Zinc fingers comprise more than 50 unique domains that are encoded within > 5% of human genes. Zinc finger domains bind diverse biomolecules including DNA, RNA, lipids, proteins including histone marks (methylation), small-protein modifiers ubiquitin and SUMO, as well as poly(ADP-ribose).
- Many DNA damage factors and telomere-associated proteins contain one or more ZnFs. Recent work has identified numerous new proteins containing ZnFs that orchestrate interactions involved in genome integrity pathways.
- The integral association of DNA, chromatin and signaling pathways involving protein-protein interactions and PTMs in genome maintenance highlights the need for zinc finger domain-containing proteins in mediating and regulating these interactions.

Figure 1 (Key Figure). Macromolecular binding specificities of zinc finger domains
(A) Zinc finger domains bind several types of substrates in addition to nucleic acids. (B) List of zinc finger domains and their binding substrates.

Figure 2. ZNF domains involved in the ZMYND8-mediated DDR pathway

Binding of PARP to DNA DSBs through its PARP ZNF domain promotes recruitment of KDM5A. The ZNF-containing PHD domain of KDM5A recognizes and promotes the demethylation of H3K4me3 near DSBs, allowing ZMYND8 to bind to acetylated chromatin and DNA through its PHD-BRD-PWWP and ZNF domains respectively. The ZNF MYND domain of ZMYND8 interacts with the PPPLΦ motif of NuRD subunit GATAD2A, recruiting the NuRD complex to damage sites to promote repression of active transcription and DNA repair of DSBs. ZMYND8 interacts with a complex containing ZNF532, ZNF592 and ZNF687, which also associate with damage sites and regulate this pathway.

Figure 3. ZNF proteins and telomere maintenance

Telomere length is determined by the balance between telomere erosion and telomere elongation processes. The ZNF-containing proteins TZAP, ZNF827 and ZSCAN4 play key roles in promoting telomere homeostasis under various physiological conditions (see text for details).

Table 1
Zinc finger proteins and genome stability

Specific zinc finger domains are listed along with number of genes containing each ZnF domain. Number of genes implicated in genome instability from each domain are provided, along with key examples and references.

Domain Name	Number of Genes	Genes Implicated in Genome Stability	Key Examples	References
3CxxC	7	0		
A20	5	0		
AD-type	1	0		
ADD-type	4	1	ATRX	[80]
AN1-type	8	1		
B-Box	54	4	PML, KAP1 (TRIM28)	[81], [82]
BTB/POZ	154	12	BACH1, ZBTB48 (TZAP)	[83], [77]
C1_4	1	0		
C2C2	6	1	TCEA	[26]
C2CH	13	0		
C2H2	681	37	ZBTB48 (TZAP), APTX, RNF138, ZATT	[77], [32], [84], [85], [37]
CCHC	55	2	TOP3A	[86]
C3HC	2	0		
CCCH	46	2	RNF113A, ZC3H11A	[26]
C4	56	2	ESR2, NR1H4	[26]
CXXC	13	1	KDM2A	[87]
C2HC5	1	0		
C5HC2	24	3	KDM2A, KDM4D, KDM5A	[87], [88], [61],
CHHC	3	0		
CTCHY	1	1	PIRH2	[89]
CW	7	0		
DBF	3	0		
DNA-Directed DNA Polymerase, Family B, Alpha	1	1	POLA1	[90]
Extended PHD (ePHD)	23	0		
FCS Type	5	0		
FLYWCH	1	0		
FYVE	32	1	ZFYVE26 (SPG15)	[91]
GATA	12	0		
HIT	6	1	INO80B (ZNHIT4)	[92]
KRAB	309	4	ZNF829	[26]
LIM	71	1	CRIP1	[93]
MIZ	7	2	PIAS4, MMS21	[49], [70]

Domain Name	Number of Genes	Genes Implicated in Genome Stability	Key Examples	References
MYM	6	1	ZMYM3	[46]
MYND	21	2	ZMYND8	[58]
PARP	2	2	PARP1, LIG3	[94], [95]
PBZ (C2H2 APLF-like)	2	2	APLF, CHFR	[69]
PHD	78	15	BAZ1A, KDM5A, KAP1 (TRIM28)	[96], [61], [82]
Rad18	6	6	SPRTN, POLK, RAD18, SNM1A	[64–66, 97]
RBZ (RanBP2)	23	6	NEIL3, ZRANB3	[67, 98]
RING	247	29	BRCA1, RNF8, RNF138, RNF168, KAP1 (TRIM28)	[99], [100], [101], [82], [7], [8], [102], [103]
SCA7	6	0		
Sec23/Sec24 ZNF	6	0		
SWIM	10	1	ZSWIM7 (SWS1)	[104]
TAZ	2	2	CBP, p300	[71]
TFIIB	4	0		
TRAF	23	1	TRAF6	[105]
Translation Initiation Factor IF2/IF5	2	0		
TTF	4	0		
UBP	14	5	USP51, USP16, USP3	[68], [30], [106]
ZPR1	1	0		
ZZ	20	3	HERC2, CBP, p300	[107], [71]