New factors in mammalian DNA repair—the chromatin connection

G Raschellà1, G Melino2,3 and M Malewicz2

In response to DNA damage mammalian cells activate a complex network of stress response pathways collectively termed DNA damage response (DDR). DDR involves a temporary arrest of the cell cycle to allow for the repair of the damage. DDR also attenuates gene expression by silencing global transcription and translation. Main function of DDR is, however, to prevent the fixation of debilitating changes to DNA by activation of various DNA repair pathways. Proper execution of DDR requires careful coordination between these interdependent cellular responses. Deregulation of some aspects of DDR orchestration is potentially pathological and could lead to various undesired outcomes such as DNA translocations, cellular transformation or acute cell death. It is thus critical to understand the regulation of DDR in cells especially in the light of a strong linkage between the DDR impairment and the occurrence of common human diseases such as cancer. In this review we focus on recent advances in understanding of mammalian DNA repair regulation and a on the function of PAXX/c9orf142 and ZNF281 proteins that recently had been discovered to play a role in that process. We focus on regulation of double-strand DNA break (DSB) repair via the non-homologous end joining pathway, as unrepaired DSBs are the primary cause of pathological cellular states after DNA damage. Interestingly these new factors operate at the level of chromatin, which reinforces a notion of a central role of chromatin structure in the regulation of cellular DDR.

INTRODUCTION

Cellular DNA is undergoing a constant damage due to the action of endogenous free radicals, replication errors and exogenous sources of radiation.1 Cells have evolved a sophisticated set of DNA damage sensors that serve to detect DNA damage and launch activation of several stress response pathways collectively referred to as the DNA damage response (DDR).2 To prevent deleterious consequences of replication through damaged DNA, cells typically temporarily arrest cell cycle (checkpoint activation).3–5 Inhibiting transcription and translation and initiate DNA repair. At the face of very extensive DNA damage cells can also activate a program of self-destruction termed apoptosis.6,7 Persistent DNA damage on the other hand can lead to cellular senescence.7 The ultimate cell fate following DNA damage will be determined by the extent, type and severity of DNA damage experienced by the cell. The efficiency of DNA repair plays a paramount importance in cellular response to DNA damage induced by drugs that are frequently used in cancer chemotherapy.8–13 The repair of DNA requires lesion recognition by specialized sensor molecules but also importantly adjustment of chromatin structure in the lesion vicinity.14 This DNA damage-induced chromatin changes serve several purposes. Marking of chromatin at the lesion creates a recognition signal for the cellular machinery to assemble at the site to initiate the repair process. Secondly chromatin changes at the DNA facilitate the repair process by allowing access of various repair and signaling complexes. Thus chromatin-level responses lie at the heart of cellular coordination of the DDR.15 Chromatin changes in response to DNA damage occur at several distinct levels such as: nucleosome remodeling, variant histone exchange, non-histone chromatin protein mobility alteration and histone tail post-translational modification.16 In this review we will summarize several recent findings of new protein factors and mechanisms governing the regulation of cellular DNA repair with emphasis on double-strand DNA break (DSB) repair and highlighting the central role of chromatin-level regulation (Figure 1).

NEW FINDINGS IN DOUBLE-STRAND DNA BREAK REPAIR REGULATION—FOCUS ON NON-HOMOLOGOUS END JOINING

Although DNA can be damaged in several distinct ways the formation of DSBs is considered the most dangerous to the cell.17 This is due to complexity of the DSB repair process and a potentially dramatic inhibition of replication and transcription the DSBs impose. There are two general strategies mammalian cells utilize to deal with double-strand breaks in DNA: non-homologous end joining (NHEJ) and homology-directed repair.18,19 Here we focus on the recent progress in understanding NHEJ mechanism and direct those interested in learning the latest on homology-directed repair regulation to excellent recent reviews published elsewhere.20 NHEJ repair process is aimed at restoration of the linear DNA structure across the break with the accuracy of the repair playing a less critical role.21,22 A specialized DSB sensor Ku70/Ku80 heterodimer, which has a high affinity for double-stranded DNA, is recruited to DSBs within seconds to initiate the classical NHEJ repair process.3 Binding to DNA induces an

1 ENEA Research Center Casaccia, Laboratory of Biosafety and Risk Assessment, Rome, Italy; 2 Department of Experimental Medicine & Surgery, University of Rome Tor Vergata, Rome, Italy and 3 MRC Toxicology Unit, Hodgkin Building, Leicester, UK. Correspondence: Dr M Malewicz, MRC Toxicology Unit, Hodgkin Building, Lancaster Road, Leicester LE1 9HN, UK.
E-mail: mm531@le.ac.uk
Received 1 December 2016; revised 1 February 2017; accepted 4 February 2017; published online 10 April 2017
allosteric change within the Ku complex, which results in recruitment of the DNA-PKcs protein. DNA-PKcs has a protein kinase activity and is activated by the presence of free DNA ends. Collectively Ku/DNA-PKcs complex is called the DNA-PK kinase and its activity is triggered by DSBs. DNA-PK bound to DSBs orchestrates the DNA repair process by allowing DNA end modification enzymes to access to DSBs to generate ligatable DNA ends (Figure 2). Additional core components of NHEJ are XLF/Cerunnos and XRCC4/Ligase IV complex, which are recruited to DNA in Ku-dependent fashion. Independently of Ku interaction, XLF and XRCC4 also are able to form long filamentous assemblies at DSBs, which contribute to proper DSB alignment, whereas Ligase IV is responsible for the final ligation reaction.23,24

XLF/Cerunnos was identified in the year 200625,26 and was considered to be the final core component of NHEJ to be discovered. It was thus remarkable that in 2015 three independent papers reported a discovery of new NHEJ factor—c9orf142—subsequently named PAXX (paralogue of XRCC4 and XLF)27,28 or XRCC4-like small protein.29 It remains unclear as to why PAXX factor had not been found earlier, however, a possibility exist that it plays a minor role in rodent cell lines, which were the foundation for the early NHEJ characterization efforts.30 Clearly humans evolved a more prominent role for some NHEJ proteins highlighted by essential functions for Ku and DNA-PKcs in human cells.31 The discovery of PAXX uncovered an unforeseen complexity in NHEJ activity regulation. Although PAXX tertiary (but not primary) structure resembles that of both XLF and XRCC4 and thus is considered their paralog (Figure 3), PAXX actually associates with DNA–PK complex (through specific contacts with Ku subunit; Figure 2). Furthermore evolutionary presence of PAXX is limited and, unlike XLF and XRCC4, it is not found in fungi, worms and the fruit flies. The fact that PAXX primary structure is most conserved in vertebrates (some simpler organism bear a more diverged homolog of PAXX27–29) suggested an important role for this protein in somatic DNA recombination. Two recent papers confirmed this prediction by showing that in pro-B cells PAXX is essential for variable, diversity, joining recombination, although surprisingly its function is normally masked by the dominant activity of XLF.32,33 Accordingly unlike the single knockouts PAXX/XLF double knockout B cells have a dramatic defect in variable, diversity, joining recombination. Furthermore generation of PAXX/XLF double knockout mice have been recently reported.34 Similarly to both XRCC4 and Ligase IV, single-knockout mice PAXX/XLF-combined mutant animals were not viable demonstrating important yet overlapping roles for PAXX and XLF in mammalian NHEJ. These findings explain a long-standing puzzle of a relatively weak phenotype associated with XLF loss.35 Thus it appears that PAXX and XLF have partially overlapping and redundant function in mammalian DSB repair. Based on in vitro studies and human cellular models PAXX functions by stimulating the activity of Ligase IV in promoting blunt-end DNA ligation and in conjunction with XLF stimulates the non-compatible (gapped) DNA end ligation.28 Both activities are strictly dependent on direct contacts between the conserved PAXX C-terminus and the Ku DNA sensor. PAXX interacts with DNA-bound Ku, however, the presence of DNA also triggers interaction of Ku with all other NHEJ

Figure 1. A simplified scheme of chromatin changes in response to double-strand break (DSB) occurrence highlighting a central role of the ATM kinase. DSBs are recognized by the MRN complex (MRE11; RAD50; NBS1), which leads to activation of the ATM kinase.111 Interaction between ATM and Tip60 is facilitated by FOXO3A transcription factor.30 Binding of Tip60 to methylated histones (predominantly H3K9me3) is required for its action on ATM and other histone substrates (e.g., H4). Tip60 acetylates histones leading to the formation of open relaxed chromatin structure.44 This step is facilitated by p400 histone chaperone-mediated variant histone exchange at DSBs (H2A.X is replaced by H2A.Z) occurring in close proximity to DSBs (up to ca 3.5 kb away from a DSB).39 The main substrate of ATM kinase is histone H2A.Z and phospho-H2A.Z (termed γH2A.Z) spreads away from the DSBs into megabase sized domains.113 Phospho-H2A.Z is recognized by MDC1 adapter protein, which is also the substrate of ATM.114,115 Phospho-MDC1 in turn recruits RNFLRZ ubiquitin ligase.116 Linker histone H1 is the main substrate of RNFLRZ and ubiquitinated H1 is recognized by RNFL8 ubiquitin ligase.17 RNFL8 ubiquitiniates histone H2A.Z on K13/15, which facilitates recruitment of the key adapter protein 53BP1.118 Stable binding of 53BP1 also requires its association with methylated histone H4 (K20me2).119,120 Of note histone methylation is a constitutive chromatin mark, whereas both histone acetylation and ubiquitination are dynamic DNA damage-induced modifications.121 53BP1 serves as a critical regulation of DSB repair pathway choice and promotes NHEJ repair by inhibiting DNA resection (a critical step in homologous recombination (HR) repair).122 ATM is also implicated in the repair of heterochromatin DSBs by phosphorylating KAP1 protein, which in turn promotes opening of heterochromatin to allow for the access of the DNA repair machinery.123

Oncogene (2017) 4673 – 4681
Figure 2. A simplified model for DSB repair mechanism via non-homologous end joining (c-NHEJ) in chromatin with emphasis on the role of poly-ADP-ribosylation (PAR). Double-strand DNA break (DSB) is initially bound by Ku dimer. DNA-bound Ku undergoes an allosteric change to recruit other effectors of the c-NHEJ DSB repair pathway: DNA-PKcs, PAXX, XLF and XRCC4 Ligase IV. Ku can potentially spread away from the DSB. The interaction between Ku and DNA-PKcs is further stabilized by various factors such as LRF and LIMP (ncRNA). This figure includes some of the known chromatin-remodeling steps that occur at DSBs such as generation of limited nucleosome-free region and PAR-dependent variant histone exchange (see Figure 1 for a more extensive description of DSB-related chromatin changes). PAR-dependent H3.3 variant histone exchange is promoted by PARP-1 in conjunction with the CHD2 chromatin remodeler and is required for stable association of Ku with DSBs. Additional accessory c-NHEJ factor is the APLF protein that binds to PAR-sylated chromatin (in this case PAR-sylation is catalysed by PARP3) and interacts with Ku to enhance the stability of repair complexes on chromatin. NR4A nuclear orphan receptors bind DNA-PKcs and are able to promote DSB repair.

Figure 3. Newly discovered c-NHEJ factor c9orf142/PAXX structurally resembles XRCC4. Figure shows computer models of XLF, XRCC4 and c9orf142/PAXX (adapted from Craxton et al., 2015). These proteins typically form dimers, however, here for simplicity and to highlight their structural similarity monomers are depicted. For XLF C-terminal portion of the coiled-coil has been shortened. The overall structure of a typical XRCC4 paralog includes a globular head domain, a centrally located coiled-coil and the C-terminal region (CTR, not shown). The CTRs are intrinsically disordered and not visible on available crystal structures. The head domain of XLF and XRCC4 is responsible for the formation of XLF/XRCC4 filaments, whereas the head domain of PAXX has an unknown function. The coiled-coils include regions necessary for dimerization. CTRs of XLF and PAXX mediate the interaction with Ku (not shown).
DNA breaks are also detected by several poly-ADP-ribosylases41,42 role of poly-ADP-ribosylation in this process. Accordingly, cellular present not entirely clear several observations point to a possible determining the extent of Ku loading on chromatin? While at cells appears to be regulated. What are the mechanisms of the protein stoichiometry involved. It thus currently unclear of the relation to Ku chromatin binding nor are such assays informative neither of these assays provides DSB distance information in

THE IMPORTANCE OF CHROMATIN FOR NHEJ REGULATION

Early biochemical data obtained in vitro with pure proteins and naked DNA suggested a simple 1:1 Ku/DNA end binding stoichiometry.36 This model had recently been validated in vivo by a discovery of small Ku foci assembling on DSBs in irradiated cells.37 However, other reports employing chromatin immunoprecipitation observed a spread of Ku (and of DNA-PKcs)48 up to 5 kb away from the break on chromatin flanking DSBs.39 The discrepancy likely lies in the methodology, as the visualization of small Ku foci is only possible once the majority of chromatin-bound Ku is pre-extracted prior to fixation, which leaves a possibility that some RNAse-sensitive fraction of Ku is able to spread away from the DSB. DNA-damage-induced Ku chromatin loading can also be observed with cellular fractionation techniques27 and via laser based microirradiation.40 However, neither of these assays provides DSB distance information in relation to Ku chromatin binding nor are such assays informative of the protein stoichiometry involved. It thus currently unclear of whether Ku can spread on chromatin at DSBs. However, in contrast to simple Ku/DSB interaction model derived from in vitro studies the loading and maintenance of Ku on chromatin in living cells appears to be regulated. What are the mechanisms determining the extent of Ku loading on chromatin? While at present not entirely clear several observations point to a possible role of poly-ADP-ribosylation in this process. Accordingly, cellular DNA breaks are also detected by several poly-ADP-ribosylases41,42 (PARPs; most notably PARP1, 2 and 3)49 and binding of these enzymes to DNA strand breaks triggers their activation with subsequent catalysis of long poly-ADP-ribose (PAR) chains at the ribose units and therefore PAR has a certain chemical resemblance to both RNA and DNA. PAR can be covalently attached to various proteins at DNA lesions with histones being the most prominent target,44 however, the exact amino acid positions of this modification on histone proteins had not been precisely mapped. PAR polymer is specifically recognized by specialized protein domains present in various DNA repair factors such as the APLF protein,45,46 which performs an accessory/non-essential role in mammalian NHEJ reactions. In the context of DSB repair APLF had been shown to contribute the NHEJ execution at the chromatin level.47 APLF utilizes a PAR-binding zinc-finger (ZF) domain to bind PAR and a centrally located MID domain to bind Ku.47 By coupling these abilities APLF acts as a scaffolding molecule able to enhance the stability of NHEJ repair complexes in the context of PARylated chromatin. A strong support for a major PAR contribution to NHEJ comes from studies in the slime mold Dictyostelium discoideum.48

This model organism apparently lacks an APLF homolog, however, its Ku70 homolog bears a PAR-binding ZF domain, which is essential for Ku functionality in Dd NHEJ. Of note, in Dictyostelium the APLF function may be partly taken over by other proteins that possess additional domains of significant homology to human APLF, namely XRCC149 and APL.50 Despite these findings in mammalian DSB repair models the contribution of PARP1 to classical NHEJ had been a subject to an intense debate.43 The main argument against the role of PARP1 in NHEJ was a relatively minor phenotype of PARP1 mutant mice in terms of radiation sensitivity and DSB repair impairment and inconsistent phenotypes associated with PARP-1 deficiency observed in cellular settings.51 Furthermore, a strong case emerged for a participation of PARP1 in an alternative form of NHEJ,52 which operates under circumstances of defective or suppressed classical NHEJ. Notably the latest findings suggest a possible mechanism of PARP1 contribution to classical NHEJ.52 Accordingly this model proposes a PARP1/CHD2/H3.3-dependent chromatin-remodeling step being necessary for proper Ku and XRCC4 loading on chromatin at DSBs. Such a model is consistent with data obtained using DSB inducing enzymes, where a certain level of nucleosome disruption at DSBs had been found necessary for XRCC4 loading.53 The definition of PARP-1 function in c-NHEJ explains several independent observations linking PAR, PARP1 and Ku in classical NHEJ regulation in human cells (Figure 2). There is evidence for other PAR-dependent mechanisms operating in mammalian DSB repair,44,55 however, at present the exact relationship of these factors to known DNA repair pathways awaits further clarification. Finally Ku/chromatin interaction is subject to regulation via chromatin acetylation and specific nucleosome remodeling at DSBs.39,40,56 In summary although the exact Ku stoichiometry at DSBs and the neighboring chromatin is still uncertain the binding of Ku to DNA lesions in chromatin can be regulated by a variety of factors.

Similarly to Ku chromatin recruitment Ku/DNA-PKcs complex formation at DSBs also appears regulated at various levels. For example an IncRNA molecule (LINC-P1)1 recently had been described that binds to Ku80 and facilitates its interaction with DNA-PKcs.57 Not of note as mentioned above the method for detection of Ku foci involves a RNAse treatment step,37 which raises a question of whether the bulk of chromatin-bound Ku is retained at this structure via specific RNA-Ku interactions. Other examples of regulation of this step of the repair complex assembly include a case of sequence-specific transcription factor (TF) LRF.58 It has been demonstrated that LRF directly binds DNA-PKcs protein and somehow increases its association with Ku contributing to maintenance of proper levels and stoichiometry of DNA-PK holoenzyme on chromatin. Strikingly in the genetic absence of LRF, the overall kinase activity of DNA-PK is diminished, which is a dramatic finding given a large excess of Ku and DNA-PKcs levels in cells. A potential explanation for this is that the rodent cell culture system (mouse embryonic fibroblasts) used in that study is characterized by lower steady-state DNA-PK levels than an average human cell.59 Under these specific circumstances efficient DNA-PK activation in vivo might require additional accessory factors, which could be a subject to additional signal- or tissue-specific control. Interestingly, a similar regulatory model of DNA-damage kinase activation with participation of bridging factors in a form of sequence-specific transcriptional regulators have recently also been described for ataxia telangiectasia-mutated (ATM) kinase.60 ATM is a very prominent DDR-activated kinase that functions primarily in signal transduction and DSB repair.61 ATM activity is vital for several aspects of DDR regulation.62 Most notable is its ability to promote various DSB repair pathways such as homologous recombination (through phosphorylation of a key resection factor Ctp163), the repair of DSBs in heterochromatin (by phosphorylation of KAP1 protein64) and c-NHEJ (in that process ATM is redundant with XLF65). ATM is also very prominent in regulating DSB-related signaling (including checkpoint induction), chromatin remodeling and histone tail post-translational modifications (Figure 1). Recently FOXO3A TF had been found to promote association of ATM with TIP60 acetyltransferase (Figure 1), which is one of the many avenues for optimal ATM activation after DNA damage.66 Interestingly NR4A nuclear orphan receptors can also bind and collaborate with DNA-PKcs in DSB repair thus expanding the list examples of TFs involved directly in DNA repair.64 Collectively these observations suggest that a larger

Oncogene (2017) 4673–4681
collection of regulatory factors exist in living cells to control the activity and assembly of protein complexes containing DNA damage-activated protein kinases. Interestingly the above mentioned mechanisms seem most relevant in vivo in the native chromatin context (summarized in Figures 1 and 2), which highlights the need for more extensive efforts to study the DNA repair regulation under physiological conditions in chromatin whenever possible.

CONTRIBUTION OF TRANSCRIPTION FACTORS TO DDR REGULATION.

Importantly, however, several prominent DDR factors have a capacity to regulate transcription and contribute to genomic stability and other processes indirectly via that mechanism. Examples are numerous and include such key DDR proteins such as S3BP1,66 BRCA1,67 CtIP,68 KAP1,69 PTP,70,71 DNA-PKcs,72 ATM,73 ATM1,74 CHD475 and many others. Reciprocally several TFs that evolved to control gene expression have been increasingly associated with direct regulation of DDR related processes at DNA lesions76,77 (see above for LRP58). With regard to the DDR regulation a given protein factor can operate both at the lesion and through indirect means by affecting transcription of direct effectors.76,77 Interestingly for many of such multifunctional factors their direct function in DDR and in transcription can be separated genetically and involve distinct domains and interaction partners (S3BP1 being an excellent example66). Interestingly a recent study of TF recruitment to DNA damage sites further blurred the division between a transcriptional regulation and a direct action at DNA lesions by showing that some TFs use their DNA-binding domain for interaction with DNA exposed at the damaged break.78

TFs play a central role in controlling virtually all biological processes occurring in the cells including cell cycle progression,79 maintenance of intracellular metabolic and physiological balance, cellular differentiation, developmental time courses79,89 and DNA repair.80 Surprisingly, the exact number of human TFs remains still undefined. This lack of knowledge depends at least in part, on the definition of TF that varies in different studies attempting to catalog this class of genes.81,82 For instance, transcription co-factors (that is, proteins which modulate the activity of other transcription controllers but are unable to act independently), are not always registered in the list of ‘real’ TFs. Vaquerizas et al. utilized the definition of TFs as a class of proteins that binds DNA in a sequencespecific manner, but are not enzymatic or do not form part of the core initiation complex.83 According to this classification, the Authors listed a high confidence dataset of approximately 1400 genomic loci that encode TFs. This number is expected to increase to an upper bound of 1700–1900 TF-coding genes in the human genome.

A distinctive part in the architecture of TFs is the DNA-binding domain that has been utilized for their classification. The most represented DNA-binding domain among human TFs is the two-cysteine two-histidine ZF followed by homeodomain and helix-loop-helix.86 Some types of DNA-binding domains are frequently used in the cell for the execution of specific functions. For example, the homeobox domains are essential for morphogenesis, organogenesis and establishment of body plan in vertebrates.87,88 Notwithstanding the evolutionary conserved nature of these TF families in vertebrates, the two-cysteine two-histidine ZF family stands out as a prominent exception. In fact, many ZF factors derive from duplication throughout the evolution.89 Consequently, this evolutionary dynamics contributes to their difficult classification in functionally distinct subfamilies. Typically, ZF factors possess an array of two-cysteine two-histidine motifs that defines a polyadactyl structure in which each finger binds three adjacent nucleotides at the DNA recognition sites. The polyadactyl structure further complicates the ZF factors binding specificity since adjacent motifs influence each other’s DNA binding.89

Recently, it has been demonstrated that the ZF factor Zfp335 possesses two major DNA-binding domains comprising distinct ZF clusters.90 Intriguingly, each domain encodes a different sequence specificity implying that binding to multiple sequence motifs could be relevant for specific gene regulation. Usage of multiple motifs may result in a more efficient targeting of TFs to their binding sites and could be important for context-dependent function. Thus, ZF factors are expected to have a broad number of targets whose functional significance is strictly dependent on their binding topography, which in turn can vary depending on the physiological state of the cell. Indeed, ZF factors that were initially defined as specific for regulation of one pathway, to a deeper analysis, revealed a more pleiotropic function. This is the case of CTCF, an 11 ZF protein initially described as a negative regulator of Myc expression.91 Further analysis highlighted a much broader ability of CTCF in recognizing different targets through the combinatorial use of its 11 ZFs. In vivo mapping revealed that CTCF reads sequence diversity through ZF clustering. In fact, ZFs 4–7 anchor CTCF to most of the targets containing the core motif while ZFs 1–2 and ZFs 8–11 clusters recognize non-conserved flanking sequences.92 As a consequence of this combinatorial modality of recognition, the targets of a single-ZF factor often refer to a variety of cellular pathways, which are not obviously interrelated.

The plasticity of TFs in recognizing their targets highlighted another somehow unexpected ability of this broad family of genes. As mentioned earlier, a recent study that utilized epitope-tagged proteins for localization to sites of DNA damaged by UV laser microirradiation, found >120 proteins that localize to damaged chromatin. Intriguingly, ~70% of the TFs included in this study, were able to migrate to damaged DNA.78 Of interest, in a set of 35 TFs evaluated for their presence at the DNA-damaged sites, 13 were ZF factors. The integrity of the DNA-binding domain seems to be a necessary condition for recognition of damaged DNA by TFs. Relocation of the TFs at the DNA-damaged sites relies on chromatin decompaction, which in turn is dependent on PARP1 activity. The mechanism(s) through which PARP1 decompacts chromatin until recently were completely unclear. One example recently emerged in the context of NHEJ repair (see above95). Another possible mechanism could be that PARP1 somehow recruits other chromatin remodelers, however, silencing of remodelers such ALC1, CHD4, INO80, TIP60, KAT2A, SMARCC1, SMARCC2, BAZ1B, EZH2, SUZ12 and p300 did not affect recruitment of TFs on damaged DNA. This result does not rule out the existence of a redundant mechanism, which implies the synergistic action of a combination of factors. Alternatively, PARP1-dependent chromatin remodeling could be dependent on a yet unidentified factor. Since some TFs are endowed with the ability of recruiting chromatin remodelers,93 a direct contribution of TFs to this task can also be hypothesized at the DNA-damaged sites.

ZNF281 A MULTI-TASK ZINC FINGER PROTEIN

The molecular structure of ZNF281 (aliases: ZBP-99, ZNP-99) is characterized by 4 two-cysteine two-histidine ZFs located near the N-terminal of the protein which has a mass of 99 kD (Figure 4). The prominent functional feature of ZNF281 resides in its ability to bind to GC-rich sequences located in the regulatory regions of many genes where its binding often results in transcriptional promotion or repression of the targets.94,95 After approximately a decade during which its function(s) in the cell remained undetermined, zfp281 (the mouse homolog of ZNF281) was recognized as a component of a protein interaction network for pluripotency of embryonic stem cells.96 Further analysis of its role demonstrated that zfp281 is a transcriptional repressor of Nanog, an essential gene for cellular stemness.97,98 Indeed, genetically ablated Zfp281 null ESCs unambiguously argue for a repressor
function of Zfp281 in regulation of major stem cell factors among which Sox2 and Oct4. Of interest, zfp281 exerts its inhibitory activity by recruiting the NuRD repressor complex on Nanog promoter. The latter mechanism was also demonstrated in the transcriptional repression of the TET2 gene. In this context, zfp281 recruits HDAC2 (a component of the NuRD complex) causing histone H3 deacetylation and chromatin compaction. In addition, zfp281 also promotes the transcription of miR302/267, which in turn post-transcriptionally represses TET2 expression. The overall result of TET2 repression is the maintenance of a state of transcriptional repression of the TET2 gene. In this context, zfp281 can regulate positively or negatively a number of target genes (blue arrows in the lower part of panel b). Zfp281 and its murine homolog zfp281 are involved in the induction of epithelial–mesenchymal transition (EMT) of colon cancer cells, in control of cellular stemness, in the inactivated expression of the Meg3 polycistron and in regulation of genes involved in the DNA-damage response (DDR). Transcription repression of Nanog and Tet2 is achieved through recruitment of the histone deacetylase HDAC2. Arrow-headed and brackeled lines indicate activation and inhibition respectively.

**ZNF281 IN THE DNA DAMAGE RESPONSE**

We recently reported an increase of ZNF281 expression after genotoxic stress by DNA damage inducing drugs. We observed this phenomenon in p53-proficient and -deficient tumor cells, as well as in normal primary keratinocytes and in mouse skin in vivo. The increase of ZNF281 following DNA damage occurs through dominant, p53-independent mechanism, which does not rely on transcriptional regulation. A significant delay in DNA repair in cells silenced for ZNF281 expression suggests that the expression of ZNF281 could have functional implications in DNA repair. Among several DDR-associated genes whose expression is affected by ZNF281, XRCC4 and XRCC2 two components of the NHEJ and homologous recombination DNA repair pathways respectively, Chromatin immunoprecipitation followed by sequencing analysis demonstrated that Sox4 binds to DNA sequences in the proximity of ZNF281 gene, suggesting a binding-dependent regulation. The expression of ZNF281 is post-transcriptionally repressed by miR34a through a p53-dependent mechanism and by miR203. Regulation of ZNF281 could occur also through epigenetic mechanisms since differential methylation of ZNF281 gene has been recently described. Of interest, the ZNF281 protein is phosphorylated by ATM and ATM-and ataxia telangiectasia Rad3-related (ATR) kinases after DNA damage. In humans, the closest ZNF281 homolog is ZBP-89, which is frequently over-expressed in human cancer cells, where it can efficiently induce apoptosis through p53-dependent and -independent mechanisms. A human ZBP-89 splice isoform ZBP-89-DN, which lacks amino terminal residues 1–127 of the full length protein, has also been identified, which predisposes the colon to colitis.
are promoted by ZNF281 through a mechanism dependent on its binding to their promoters. Of interest, Nucleolin, a protein that mediates nucleosome disruption critical for DNA double-strand break repair is activated and which is a known target of c-Myc, is not transcriptionally activated by ZNF281, which acts instead as a co-factor of c-Myc in Nucleolin activation. Together these results demonstrate for the first time, that ZNF281 is involved in the DDR by its classical function of TF that controls the expression of other genes (Figure 4b). Nevertheless, ZNF281 could give its contribution to DNA repair through a less obvious mechanism. As mentioned before, among the TFs that were demonstrated to move to the sites of damaged DNA there is ZNF281.78 What could be the function of ZNF281 on the sites of damaged DNA? ZNF281 could recruit chromatin remodelers to the sites of broken DNA by a mechanism similar to that utilized on the promoters of Nanog53 and TET299 (Figure 4b). We can also speculate that ZNF281 interacts with core components of the DNA repair machinery and contribute to the role of the recently characterized XLK and PAXX in maintaining appropriate levels of all other repair factors on chromatin. Although our knowledge on the involvement of ZNF281 in NHEJ as well as other repair pathways is still limited, future work could discuss other relevant and unexpected roles for this factor in DNA repair.

CONCLUSIONS AND FUTURE DIRECTIONS

We conclude that a number of recently discovered factors in mammalian DNA repair operate at the level of chromatin. The recent addition of proteins such PAXX and possibly ZNF281 to the overall framework of the repair mechanisms, underscores our still incomplete knowledge of the molecular details underlying the chromatin modifications which occur during the DDR. As many clinically relevant cancer treatments rely on DNA damage induction in tumor tissue a better understanding of contribution of chromatin changes to DDRs will be instrumental in further development of such therapies.

ABBREVIATIONS

C2H2, two cyssteine two histidine; Dd, Dicystostelium discoideum; DDR, DNA-damage response; DNA-PK, DNA-dependent protein kinase; DSB, double-strand breaks; EMT, epithelial–mesenchymal transition; IncRNA, long non-coding RNA; NHEJ, non-homologous end joining; PAR, poly-ADP-ribose; TFs, transcription factors; ZFs, zinc fingers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work has been supported by the Medical Research Council, UK; grants from Associazione Italiana per la Ricerca contro il Cancro (AIRC): AIRC 2014 IG15653 (to GM), AIRC Sxmilie MCO9979 (to GM) and AIRC 2011 IG11955 (to GM), Fondazione Roma malattie Non trasmissibili Cronico-Degenerative (NCD) Grant (to GM).

REFERENCES

1. Li Z, Zhang W, Chen Y, Guo W, Zhang J, Tang H et al. Impaired DNA double-strand break repair contributes to the age-associated rise of genomic instability in humans. Cell Death Differ 2016; 23: 1765–1777.
2. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell 2010; 40: 179–204.
3. Bruinsma W, Apriela M, Garcia-Santesteban I, Kool J, Xu YJ, Medema RH. Inhibition of Polo-like kinase 1 during the DNA-damage response is mediated through loss of Aurora A recruitment by Bora. Oncogene 2016.
4. Gharmrasi SE, Cardoso R, Li L, Guturi KB, Bjergregaard VA, Liu Y et al. Rad54 and Mus81 cooperation promotes DNA damage repair and restrains chromosome missegregation. Oncogene 2016; 35: 4836–4845.
5. Paiva C, Godbersen JC, Berger A, Brown JR, Danilov AV. Targeting neddylation induces DNA damage and checkpoint activation and sensitizes chronic lymphocytic leukemia cells to alkylating agents. Cell Death Dis 2015; 6: e1807.
6. Barroso-Gonzalez J, Alcaur S, Suan S, Thomas L, Atkins KM, Aslan JE et al. PACS-2 mediates the ATM and NKP-attack-dependent induction of anti-apoptotic Bcl-xL in response to DNA damage. Cell Death Differ 2016; 23: 1448–1457.
7. Alam SK, Yadav VK, Bajaj S, Datta AK, Datta SK, Bhattacharya MJ et al. DNA damage-induced ephrin-B2 reverse signaling promotes chemoresistance and drives EMT in colorectal carcinoma harboring mutant p53. Cell Death Differ 2016; 23: 707–720.
8. Shah MY, Martinez-Garcia E, Phillip JM, Chamblass AB, Popovic R, Eppindza T et al. MMSET/WHSC1 enhances DNA damage repair leading to an increase in resistance to chemotherapeutic agents. Oncogene 2016; 35: 5905–5915.
9. Ozes AR, Miller DF, Ozes ON, Fang F, Liu Y, Matei D et al. NKP-attack-HOTAIR axis links DNA damage response, chemoresistance and cellular senescence in ovarian cancer. Oncogene 2016; 35: 5350–5361.
10. Muralidharan SV, Bhadury J, Nilsson LM, Green LC, McGuire KG, Nilsson JA. BET bromodomain inhibitors synergize with ATR inhibitors to induce DNA damage, apoptosis, senescence-associated secretory pathway and ER stress in Mv-114-induced lymphoma cells. Oncogene 2016; 35: 4689–4697.
11. Tong KL, Ota K, Komuro A, Ueda T, Ito A, Anne Koch C et al. Attenuated DNA damage repair delays therapy-related myeloid neoplasms in a mouse model. Cell Death Dis 2016; 7: e2401.
12. Montenegro MF, Gonzalez-Guerrero R, Sanchez-del-Campo L, Pinero-Madrona A, Cabezas-Herrera J, Rodriguez-Lopez JN. Targeting the epigenetics of the DNA damage response in breast cancer. Cell Death Dis 2016; 7: e2180.
13. Hedstrom E, Pedervin C, Fanebo J, Nordin B, Jirstrom K, Brennan DJ et al. Downregulation of the cancer susceptibility protein WRAP53beta in epithelial ovarian cancer leads to defective DNA repair and poor clinical outcome. Cell Death Dis 2015; 6: e1892.
14. Lukas J, Lukas C, Bartek J. More than just a focus: the chromatin response to DNA damage. Nat Rev Mol Cell Biol 2010; 11: 1161–1169.
15. Ceccaldi R, Rondinelli B, D’Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends Cell Biol 2016; 26: 52–64.
16. Polo SE, Jackson SP. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. Genes Dev 2011; 25: 409–433.
17. Goodarzi AA, Jeggo PA. The repair and signaling responses to DNA double-strand breaks. Adv Genet 2013; 82: 1–45.
18. Price BD, D’Andrea AD. Chromatin remodeling at DNA double-strand break. Cell 2013; 152: 1344–1354.
19. Yang Q, Pan Q, Li C, Xu Y, Wen C, Sun F. NRG1 is involved in homologous recombination repair to resist the DNA-damaging chemotherapy and composes a ternary complex with RNF8-BARD1 to promote cell survival in squamous esophageal tumorigenesis. Cell Death Dis 2016; 23: 1406–1416.
20. Prakash R, Zhang Y, Wais F, Jasim M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb Perspect Biol 2015; 7: a016600.
21. Waters CA, Strande NT, Wyatt DW, Pryor JM, Ramiden DA. Nonhomologous end joining: a good solution for bad ends. DNA Repair 2014; 17: 39–51.
22. Lieber MR. The mechanism of double-strand DNA break repair by the non-homologous DNA end-joining pathway. Annu Rev Biochem 2010; 79: 181–211.
23. Brouwer I, Sitters G, Candelli A, Heerema SJ, Heller I, de Melo AJ et al. Sliding sleeves of XRCC4-XLF bridge DNA and connect fragments of broken DNA. Nature 2016; 535: 556–569.
24. Ahrenos P, Smith P, Jackson SP. XLF interacts with the XRC4-XDNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 2006; 124: 301–313.
25. Buck D, Mallivert L, de Chasseval R, Barraud A, Fodaneche MC, Sanal O et al. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. Cell 2006; 124: 287–299.
26. Ochi T, Blackford AN, Coates J, Jhujh S, Mehmood S, Tamura N et al. PAXX, a paralog of XRC4 and XLF, interacts with Kx to promote DNA double-strand break repair. Science 2015; 347: 185–188.
27. Xing M, Yang M, Huo W, Feng F, Wei L, Jiang W et al. Interactionome analysis identifies a new parologue of XRC4 in non-homologous end joining DNA repair pathway. Nat Commun 2015; 6: 6233.
28. Cistron A, Somers J, Munnur D, Hughes-Jones R, Cain K, Malewicz M. XLS (c9orf142) is a new component of mammalian DNA double-stranded break repair. Cell Death Differ 2015; 22: 890–897.
29. Thacker J, Zdziecienka ZM. The XRCC genes: expanding roles in DNA double-strand break repair. DNA Repair 2004; 3: 1081–1090.
31 Fattah KR, Ruis BL, Hendrickson EA. Mutations to Ku reveal differences in human somatic cell lines. DNA Repair 2006; 7: 762–774.
32 Kumar V, Alt FW, Frock RL. PAXX and XLF DNA repair factors are functionally redundant in joining DNA breaks in a G1-arrested progenitor B-cell line. Proc Natl Acad Sci USA 2016; 113: 10619–10624.
33 Lescale C, Lenden Hasse H, Blackond AN, Balmus G, Bianchi JJ, Yu W et al. Spectroscopic roles of XRCC4 (poly(ADP-ribose) PAXX and XLF during VDJ) recombination. Cell Rep 2016; 16: 2967–2979.
34 Balmus G, Barros AC, Wijnhoven PW, Lescale C, Hasse HL, Boroviak K et al. Synthetic lethality between PAXX and XLF in mammalian development. Genes Dev 2016; 30: 2152–2157.
35 Li G, Alt FW, Cheng HL, Brush JW, Goff PH, Murphy MM et al. Lymphocyte-specific compensation for FcεRI/CD138 endosmosis into functions in VDJJ recombination. Mol Cell 2008; 31: 631–640.
36 Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell 1993; 72: 131–142.
37 Britton S, Coates J, Jackson SP. A new method for high-resolution imaging of Ku foc to decipher mechanisms of DNA double-strand break repair. J Cell Biol 2013; 202: 579–595.
38 Caron P, Choudriay J, Clouaire T, Bugler B, Daburon V, Aguirrebengoa M et al. Non-redundant functions of ATM and DNA-PKcs in response to DNA double-strand break repair. Cell Rep 2015; 13: 1598–1609.
39 Xu Y, Ayrapetov MK, Xu C, Gursoy-Yuzugullu O, Hu Y, Price BD. Histone H2AZ controls a critical chromatin remodelling step required for DNA double-strand break repair. Mol Cell 2012; 48: 723–733.
40 C unveil D, Gervais D, Leguet L, Polo SE, Britton S et al. Human HADC1 and HDAC2 functions in the DNA-damage response to promote DNA non-homologous end-joining. Nat Struct Mol Biol 2010; 17: 1144–1151.
41 Dantuma NP, van Attikum H. Spatiotemporal regulation of posttranslational modifications in the DNA damage response. EMBO J 2016; 35: 6–23.
42 Rulten SL, Fisher AE, Robert I, Zuma MC, Rouleau M, Ju L et al. PARP-1 and PARP-2 depolymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. Exp Cell Res 2014; 329: 18–25.
43 Gibbils-Seymour I, Fontana P, Rack JG, Ahel I. HPF1/C4orf27 Is a PARP-1-interacting protein that regulates PARP-1 ADP-ribosylation activity. Mol Cell 2014; 53: 367–375.
44 Beck C, Robert I, Reina-San-Martin B, Schreiber V, Dantzer F. Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling factor, ATMIN is a transcriptional regulator of both lung morphogenesis and ciliogenesis. J Cell Sci 2016; 129: 3845–3858.
45 Grundy GJ, Rulten SL, Zeng Z, Arribas-Boasacoma R, Iles N, Manley K et al. APLF promotes the assembly and activity of non-homologous end joining protein complexes. EMBO J 2013; 32: 112–125.
46 Couto CA, Wang HY, Green JC, Kiely R, Siddaway R, Borer C et al. PARP regulates nonhomologous end joining through retention of Ku at double-strand breaks. J Cell Biol 2011; 194: 367–375.
47 Mehta PV, Ahel D, Ryan DP, Weston R, Wiechens N et al. DNA repair factor APLF is a histone chaperone. Mol Cell 2011; 41: 46–55.
48 Guan Y, Wang G, Lee WH. CtIP, a multivalent adaptor connecting transcriptional regulation, DNA repair and cell cycle control. Proc Natl Acad Sci USA 2006; 103: 789–794.
49 Goodwin JF, Kohari V, Drake JM, Zhao S, Dygier E, Dean JL et al. DNA-PKcs-mediated transcriptional regulation drives prostate cancer progression and metastasis. Cancer Cell 2015; 28: 97–113.
50 Jang ER, Choi JD, Park MA, Jeong G, Cho H, Lee JS. ATM modulates transcription in response to histone deacetylation inhibition as part of its DNA damage response. Exp Mol Med 2010; 42: 195–204.
51 Gogoloudi P, Stevens JL, Goggolidou P, Keynton J, Wheway G, Grimes DT et al. Long noncoding RNA LINP1 et al. BRCA1 in response to histone deacetylase inhibition as part of its DNA repair. Nat Struct Mol Biol 2013; 20: 131–139.
52 Zha S, Guo C, Boboila C, Oksenych V, Cheng HL, Zhang Y et al. ATM damage response and XLF repair factor are functionally redundant in joining DNA breaks. Nature 2011; 469: 250–254.
