
Emerging Features of DNA Double-Strand Break Repair in Humans

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1. Introduction

Ionizing radiation (IR) and various cytotoxic chemicals including reactive oxygen species (ROS) induce DNA double-strand breaks (DSBs) when they attack the phosphate backbones of the two DNA strands simultaneously. DSBs, once generated, not only cause a discontinuity in the genetic code, but also are vulnerable to further loss of DNA from a nuclease attack or the formation of abnormal DNA structures from chromosomal translocation, all of which can significantly increase genomic instability leading to cancer. Repair of DSB damage is therefore crucial for maintaining the physical and genetic integrity of the genome.

DNA damage sensors are the first responder to various types of DNA damages. Upon DSB damage, Mre11–Rad50–Nbs1 (MRN) complex initially recognizes DNA damage, and recruits and activates the ataxia-telangiectasia mutated (ATM) through protein interaction with Nbs1 (Fig. 1) [1, 2]. ATM is a member of the phosphoinositide 3-kinase (PI3K)-related protein kinase (PIKK) family of serine/threonine protein kinases that phosphorylates a number of target proteins containing conserved phosphorylation motif (SQ/TQ) in response to DNA damage [3] that include MRN complex, a histone variant, H2AX, a checkpoint mediator, MDC1, a checkpoint kinase, CHK2 and p53 [4]. Phosphorylations of MRN complex, H2AX and MDC1 are necessary for recruitment of the factors involved in signal transduction and homologous recombination (HR) to facilitate the repair process [5-9]. A marginal repair defect was observed in AT cells, which could be due to the reduced efficiency of homologous recombination [10]. Damage-induced phosphorylation of CHK2 and activation of p53 also induce the cell cycle arrest at the G1 phase [6, 11, 12].

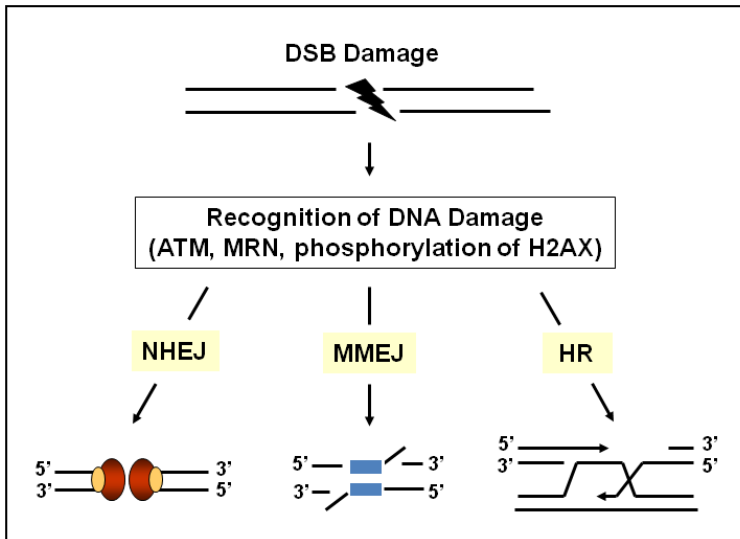


Figure 1. Three major DSB repair pathways in mammals. Following recognition of DSB damage by MRN complex and ATM, leading to phosphorylation of H2AX, DSB repair can occur through nonhomologous end joining (NHEJ), homologous recombination (HR), or microhomology-mediated end joining (MMEJ) repair pathways. The error-free pathway of HR in the late S- and G2-phases of the cell cycle requires a sister chromatid to restore broken DNA to its original sequence, whereas the error-prone pathway of NHEJ often processes the DNA by adding or deleting nucleotides before joining the ends. In some circumstances one or more of the broken ends is refractory to Ku mediated NHEJ. In this case, MMEJ can proceed by nucleolytic processing and resection of the 3'-end until a short region of complementary bases is revealed. Pairing of this microhomology stabilizes the broken ends, displaced flaps are removed and ligation can occur. Although many of the proteins involved in these major DSB repair pathways have been identified, the precise mechanisms involved remain poorly understood.

In mammals, DSB damages are largely repaired by non-homologous end joining (NHEJ) pathway throughout the cell cycle that directly ligates the break ends without the need for a homologous template (Fig. 1), so NHEJ is an error-prone repair pathway. Microhomology-mediated end joining (MMEJ) shares the repair proteins with NHEJ pathway, except that it uses a short patch (5-25 base pairs) of homologous sequences to align the broken strands before joining (Fig. 1). When a break occurs a homology of 5-25 complementary base pairs on both strands is identified and used as a basis for which to align the strands with mismatched ends. Once aligned, any overhang or mismatched bases on both strands are removed and any missing nucleotides are inserted. MMEJ works by ligating the mismatched hanging strands of DNA, removing overhanging nucleotides and filling in the missing base pairs. MMEJ repair occurs during the S-phase of the cell cycle, as opposed to the G0/G1 and early S phases in NHEJ. MMEJ ligates the DNA strands without checking for consistency and causes deletions, since it removes base pairs (flaps) on the strand in order to align the two pieces; it is an error-prone repair pathway and results in deletion mutations. In most cases, a cell uses MMEJ only when the NHEJ repair is not available or unsuitable due to the disadvantage posed by introducing dele-

tions into the genetic code. When a sister chromatid is available during late S- and G2-phases of the cell cycle, DSB damage can also be repaired by homology-directed repair, called homologous recombination (HR) (Fig. 1). This requires extensive 5'-3' resection of DNA to generate a 3' single-stranded tail. This is then displaced by the RAD51 recombinase, which forms a nucleoprotein filament which invades a homologous DNA duplex. This process named strand exchange forms a DNA crossover or Holliday junction which provides a primer to initiate new DNA synthesis. At this point there can be several outcomes. In synthesis dependent strand annealing the newly synthesized DNA reverts back to its original partner where it can be used as a template to complete repair. Alternatively for homologous recombination, the Holliday junction migrates away from the initial point of exchange (branch migration) until the junction is resolved by nucleolytic cleavage of either the crossed strands or non-crossed strands of the junction. Resolution of the two Holliday junctions in different orientations results in the exchange of flanking markers (crossover), whereas resolution in the same orientation does not result in exchange of flanking markers (non-crossover).

Since NHEJ repair involves a direct rejoining of the separated DNA ends, it requires the coordinated assembly of damage-responsive proteins at the damage site. DSB repair through NHEJ is initiated by binding Ku70-Ku80 complex to the DSB ends (Fig. 2). The Ku70/80 complex first binds to the DNA ends and recruits DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a 465-kDa ser/thr kinase that mediates synapsis of the ends and then undergoes activation of its kinase. DNA-PKcs is a member of PIKK family [13], but its contribution to checkpoint response is insignificant. Kinase activity is required for NHEJ, but its function remains unclear. Rather, it phosphorylates multiple proteins involved in NHEJ [14]. Artemis, a nuclease, and PNK, a kinase/phosphatase, process the ends [15-17], and DNA ligase IV, a complex with XRCC4, ligates two DSB ends (Fig. 2) [18, 19]. The recruitment of the XRCC4-DNA ligase IV (Lig4) complex is essential for the final step of ligation. XLF (also known as Cernunnos) is known to stimulate Lig4 *in vitro* through its interaction with XRCC4. Although DNA end joining systems in mammals are dependent on above-mentioned factors (Ku70/80, DNA-PKcs, and XRCC4/Lig4), additional factors are required for end processing during NHEJ. Artemis exists in a complex with DNA-PKcs and has nuclease activity. Mre11 and Artemis possess 3'-5' and 5'-3' exonuclease, respectively, both of which may be involved in promoting the joining of noncomplementary ends via utilizing microhomologies near the ends of the DSB. The Werner syndrome protein (WRN) with its DNA cleavage activity stimulated by Ku complex is also a potential player in DNA end processing. Others implicated in DNA end processing include FEN-1, PNK, and DNA polymerases μ and λ . In addition, DNA polymerase(s) are also likely involved in the gap filling of NHEJ reaction. Metnase (also known as SETMAR) is a new comer in DSB repair pathways that not only methylates histone H3 lysine 36 at DSB sites but also plays several other roles in the joining of DSB damages. Although this review discussed current issues on DSB repair in general, it mainly focuses on the emerging roles of Metnase in DSB repair pathway.

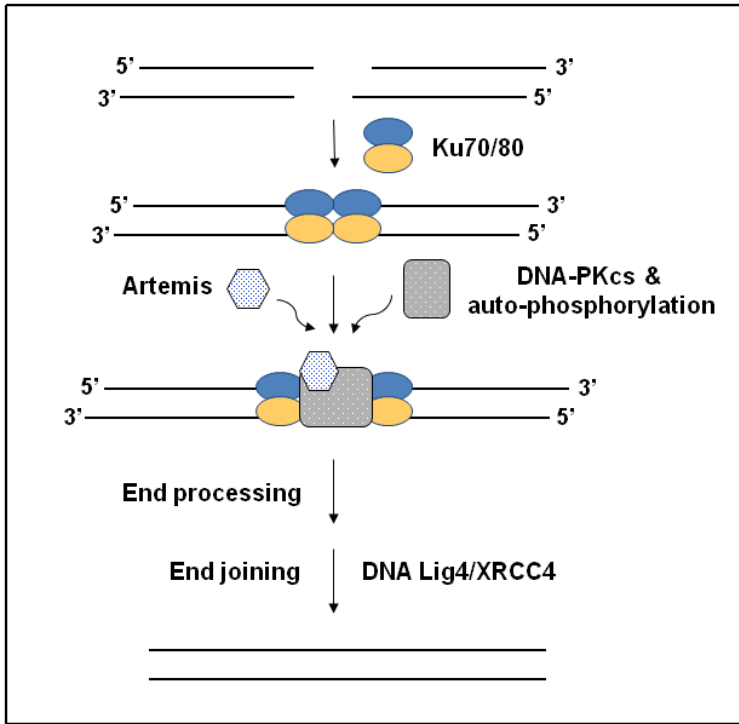


Figure 2. Nonhomologous end joining (NHEJ) repair pathway in mammals. When a DSB is introduced, Ku binds to the DNA because of its high affinity for DNA ends. The binding of Ku elicits conformational changes that allow it to bind DNA-PKcs. Ku may also serve as an alignment factor for the accuracy of NHEJ. Upon the assembly of DNA-PK on DNA breaks, this DNA repair complex activates its serine/threonine protein kinase activity and phosphorylates target substrates such as Artemis that colocalize at the ends of broken DNA prior to end processing and end joining events.

2. Human SET-Transposase chimeric protein in DSB repair

Transposases mediate DNA movement by recognizing both ends of transposon to excise the element from one site and insert it at other location in the genome, a process which can be repeated multiple times for a given segment [20-25]. It is likely that the ends are brought together and form a synaptic complex comprising two transposase molecules and the two ends of the corresponding element [25-28]. While transposase has played an important evolutionary role accounting for half of the present organization of the human genome [29], transposase activity was thought to be extinct in humans because unregulated DNA mobility could be highly deleterious in a long lived organism. To date, only one example of an intact copy of the Hsmar1 transposase domain has been identified within the human genome [30]. The Hsmar1 transposon, a class II transposable element, is an ancient element within the human genome introduced at least 50 million years ago in ancestral primates [23]. This

“functional” Hsmar1 transposase domain exists as a chimeric fusion protein, Metnase (also known as SETMAR), which resulted from an insertion of the Hsmar1 transposon downstream of a SET gene (suppressor of variegation 3-9, enhancer-of-zeste, trithorax)1 encoding a histone lysine methyltransferase (HLMT), generating the SET and transposase fusion protein [23, 30]. Metnase is not found in prosimian monkeys or other mammals. Presumably this fusion event has conferred some evolutionary advantage to anthropoid primates as the activities of both the SET domain and transposase domain have largely been retained.

The Metnase-SET domain comprises pre-SET (aa 14-118), SET (aa 120-256), and post-SET (aa 273-302) domains (Fig. 3). The pre-SET domain contains a cysteine- and histidine-rich putative Zn⁺⁺ binding motif, and the SET domain has the conserved the histone lysine methyltransferase motif shared with other SET proteins in humans [31, 32]. On the other hand, the Metnase-Transposase domain contains the conserved DNA binding and the catalytic motifs (Fig. 3). Potential DNA-binding motifs in the Metnase transposase were identified by comparative sequence analysis. These include a Nuclease-associated modular DNA-binding 1 (NUMOD1) motif, residues 417-434 representing a DNA binding helix-turn-helix based on its similarity to other families [33, 34], and a helix-turn-helix (HTH) motif, residues 347-381 (Fig. 3). Although Metnase cannot perform transposition, it has been shown to retain a number of activities associated with transposases including 5'-terminal inverted repeats (TIR)-specific DNA binding [23, 35-37], DNA looping activity [25], 5'-end processing activity [25, 35, 37], and promotion of integration at a TA dinucleotide target site [25, 38]. Recent structural analysis of the Metnase transposase domain has revealed features within the catalytic site that are distinct from those of related transposases and yet were likely present within the ancestral *Hsmar1* transposase. However, Metnase’s DNA cleavage activity, unlike other functionally active transposases, is not coupled to its TIR-specific DNA binding [35, 37].

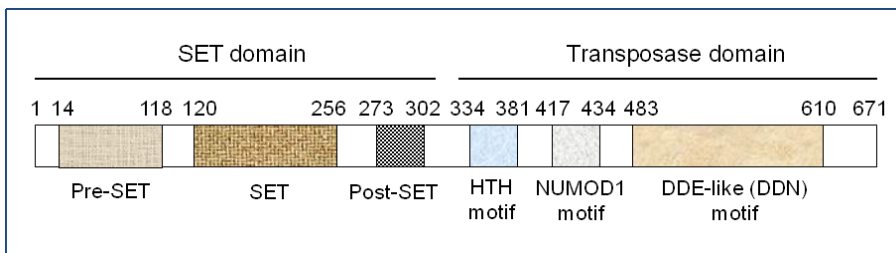


Figure 3. Schematic diagram of human Metnase (*SETMAR*). The Pre-SET domain contains cysteine- and histidine-rich putative Zn⁺⁺ binding motif. The SET domain has the HLMT motif; transposase domain contains DNA binding motifs [helix-turn-helix (HTH) and NUMOD1] and a conserved DNA cleavage (DDE-like) motif.

Metnase is widely expressed in human tissues promotes NHEJ repair and mediates genomic integration of foreign DNA [32, 35, 39]. Metnase’s involvement in NHEJ repair came from an in vivo study showing that overexpression of Metnase increased NHEJ repair, while it did not produce any significant changes in HR repair [32]. Similarly, cells treated with Metnase-specific siRNA showed a significant reduction in NHEJ repair activity in vivo. Metnase

overexpression resulted in a 3-fold survival advantage after IR treatment compared to a vector control [32], further evidence of a role for Metnase in NHEJ. Metnase is also involved in genomic integration of foreign DNA [40, 41] that depends on some of the other NHEJ factors [42, 43]. Earlier study showed that a deletion of either SET or the transposase domain abrogated Metnase's function in DNA repair, indicating that both domains are required for this function [40]. Upon DNA damage, Metnase colocalizes with other DSB repair factors and has been shown to directly interact with Pso4 [34, 36], a human homolog of the 55-kDa protein encoded by the PSO4/PRP19 gene in *Saccharomyces cerevisiae* that has pleiotropic functions in DNA recombination and error-prone repair [44-47]. Metnase-mediated stimulation of DNA end joining in vivo requires both histone methyltransferase and transposase-associated activities [32], indicating that it has multiple functions in NHEJ repair. The SET-transposase fusion protein not only promotes DSB repair, but also physically interacts with Topo II α and enhances Topo II-mediated chromosomal decatenation [24, 39], both of which are crucial for controlling DSB damage. Metnase is widely expressed, and is located at chromosome 3p26, a region of frequent abnormalities in various cancers [23, 32]. Metnase is the only known example of a protein involved in DNA repair that includes a SET domain as well as the only intact and functional *Hsmar1* transposase within the human genome.

3. Histone H3 dimethylation of Lys36 at DSB sites

DSB damage induces post-translational modification of histone proteins at the DNA damage sites, which not only is necessary for DNA damage sensing but also promotes DNA repair [48-57]. H2AX, a member of the histone H2A family, is rapidly phosphorylated in response to ionizing radiation and DNA damaging drug, generating γ H2AX [50, 53, 55-57]. Phosphorylation of the histone variant H2AX occurs at the conserved C-terminal phosphatidylinositol 3-OH-kinase-related kinase (PI3KK) motif, and likely play a key role in DDR and is required for the assembly of DNA repair proteins at the sites containing damaged chromatin as well as for activation of checkpoints proteins which arrest the cell cycle progression [58-61]. DSB damage also induces non-proteolytic ubiquitylation near DNA damage site on the chromatin. DSB-induced ubiquitination is mediated by the RNF8/RNF168 ubiquitin ligase cascade [60], and has emerged as a key mechanism for restoration of genome integrity by licensing the DSB-modified chromatin to recruit genome caretaker proteins such as 53BP1 and BRCA1 near the lesions. In parallel, Sumoylation of upstream DSB regulators is also required for execution of this ubiquitin-dependent chromatin response, although its molecular basis is not clear.

Histone methylation plays a key role in and as such regulates transcription, replication, cell differentiation, genome stability, and apoptosis [62-66]. Mounting evidence points to a role for histone lysine methylation in DSB repair [67-72]. In mammalian cells, H3K79 methylation is crucial for 53BP1 localization at DSB sites and interaction with p53 in damage checkpoint activation [73]. In *S. cerevisiae*, loss of H3K79 methylation inhibits Rad9-dependent activation of the checkpoint kinase Rad53 following DSB damage [74, 75], and in fission yeast controls the recruitment of the damage checkpoint adaptor pro-

tein, Crb2 [76-78]. A recent study showed that DSB damage induces dimethylation of histone H3 at lysine 36 (H3K36me2) in human cells [31, 32]. Chromatin immunoprecipitation (ChIP) and immunoblot analyses indicated that H3K36me2 is actually formed at DSB sites [31]. H3-K36 is associated with chromatin opening [79-84], which may also be a part of its DSB localization via chromatin modulation. In fact, mutations at known conserved SET domain amino acids (N210S, alteration at the NHSC at 210-213 to AAAA, and the YDY at 247-249 to AAA) significantly lowered DNA end joining [32]. Two conserved amino acid sequences (210-NHSCXPN-216 and 242-EEELXXXY-249) in the Metnase-SET domain are likely responsible for the interaction with SAM since a mutation at these sites failed to interact with ³H-labeled SAM [85-88]. Levels of DSB-induced H3K36me2 strongly correlate with Metnase expression and that the mutant (D248S) lacking HLMT activity fails to generate H3K36me2, suggesting that Metnase is responsible for the induction of H3K36me2 at DSB site [32]. Considering that the D248S mutant of Metnase fails to promote NHEJ repair, dimethylation of H3K36 is likely a major function of Metnase in promoting chromosomal DSB repair. Although the mechanism by which H3K36me2 promotes DSB repair is not clear, H3K36 methylation has been linked to chromatin opening accessible to transcription regulators and DNA repair proteins [89]. H3K36me2, once formed at DSB site, may create docking sites for other repair proteins, recruiting them for transcription and DNA repair. For example, H3K36 methylation attracts the histone deacetylase Rpd3S, which compact chromatin in the middle of transcribed genes, and inhibits false initiation of transcription during the elongation phase [90]. The methyltransferase Setd2 (also known as Set2) mediates trimethylation of H3K36me3 (H3K36me3), and it binds the phosphorylated tail of RNA polymerase II, implicating a role for H3K36me3 in transcription [49, 51, 91]. Setd2 mediates H3K36me3 in mammalian cells, but not di- or mono-methylation [92], raising a possibility that Metnase or other H3K36 dimethyltransferases may be necessary to generate H3K36me2 before Setd2 acts. In *Drosophila*, dimethylation of H3K36 peaks adjacent to promoters and requires distinct methyltransferases than those that mediate H3K36me3 [93]. The formation of H3K36me2 might also facilitate histone eviction at the DSB site, which then facilitates an access of the repair machineries to DNA damage site. This is supported by an observation that H3K36me2 enhances the presence of MRN complex and Ku70 at the DSB site [31]. These DNA repair proteins show an increased interaction with H3K36me2 after IR, and their presence at an induced DSB also correlated with Metnase expression levels. In addition, the chromatin immunoprecipitation study revealed that H3K36me2 not only enhances the rate of association of these repair proteins with the DSB but decreases their disassociation rates as well [31]. Because the MRN and Ku complexes can bind free DNA ends at a DSB in nonchromatinized DNA, the decreased rates of disassociation are likely the more important role of H3K36me2. This implies that the main benefit of H3K36me2 in DSB repair is more likely to stabilize the repair components at the DSB than to enhance their recruitment. It is possible that dimethylation of H3K36 at DSBs was an epiphenomenon and was not responsible for enhanced localization of early DSB repair components. On the other hand, when a point mutation at H3-K36 (K36R or K36A) caused a marked decrease in both the recruitment of NBS1 and Ku70 to the DSB and in DSB repair [31], indicating that H3K36me2 is

required for efficient assembly and retention of repair components at DSBs and for optimum DSB repair. The identification of dimethylated H3K36 as a chromatin modification that enhances DSB repair by NHEJ places this modification alongside and ubiquitylated H2A as DNA damage-induced histone modifications that recruit repair components to DSBs and enhance repair [31, 94, 95]. In this regard, H3K36me2 by Metnase is consistent with an NHEJ histone code, as defined in the original histone code hypothesis for transcriptional regulation as histone modifications, acting in a combinatorial fashion on histones, which specify unique downstream functions [56]. Previous reports indicate that histone methylation may be important in DNA DSB repair by homologous recombination: The DSB repair component 53BP1, which is required for proper homologous recombination, is recruited to sites of damage by methylated histone H3 lysine 79 (H3K79) and histone H4 lysine 20 (H4K20) [76, 78, 96]. However, neither H3K79 nor H4K20 methylation is induced by DNA damage [96]. H3K36me2 is likely reserved for NHEJ repair pathway, because Ku70 and Metnase are involved in DSB repair by NHEJ rather than HR repair and because the latter requires complete histone eviction adjacent to the DSB. Human cancer cells that express Metnase at high levels display enhanced resistance to treatment with radiation or chemotherapy [32, 131, 132]. The resistance mediated by Metnase could reflect improved stabilization of the assembly of DSB repair components at DSB sites due to the generation of H3K36me2 at these sites. If so, a targeting of Metnase's HLMT activity may improve the efficacy of common cancer therapies based on DNA damaging agents.

4. DNA endonuclease activity in the joining of DSB damage

IR induces DNA double strand breaks with different ends, most of which are not directly ligatable. Therefore, they need to be processed before end joining event in all three major DSB repair pathways, with the exception of adding nucleotides opposite to 5'-overhang by DNA polymerase [97-100]. DNA end processing can be divided into two types: ssDNA cleavage that removes either a 5'- or 3'-overhang to leave a blunt end, and nuclease activity producing a deletion that is consistent with alignment of the DNA ends by base pairing in region(s) of microhomology [17, 97, 99, 101]. Several endonucleases and their binding partners have been shown to participate in end processing during DSB repair. Mre11 and Artemis possess 3'-5' exonuclease activity and ssDNA-specific 5'-3' exonuclease, respectively, both of which may be involved in promoting the joining of noncomplementary ends via utilizing microhomologies near the ends of the DSB [17, 100, 102-106]. MRN's exonuclease activity is for mismatched DNA ends and pauses at sites of microhomology [100], while its endonuclease is to open fully paired hairpin DNA [105]. Artemis possesses an endonuclease activity specific for hairpins and 5'- or 3'-overhangs following phosphorylation by DNA-PKcs [17, 106], suggesting that it plays a role in V(D)J recombination repair and perhaps in removing the 5'- and 3'-overhangs of non-compatible ends during NHEJ repair. Human CtIP physically and functionally interacting with MRN is another player in DNA end processing [107]. CtIP was originally

identified as a binding partner for CtBP11 and the tumor suppressor proteins RB1 [108] and BRCA1 [86, 109], and is recruited to DNA damage and complexes with BRCA1 to control the G2/M DNA-damage checkpoint [110-112]. CtIP and the MRN complex promote ATR activation and HR through mediating DSB resection [107]. The Werner syndrome protein (WRN), a RecQ-like DNA helicase also possesses 3'-5' exonuclease activity [42, 43, 113]. Considering that WRN is phosphorylated by DNA-PKcs [113], and its DNA cleavage activity is stimulated by Ku complex [114], WRN could play a role in DNA end processing. Other DNA helicases such as Bloom (BLM) and DNA2 may also play a role in DNA end processing [85]. These two DNA helicases physically interact to each other to resect DNA in a process that is ATP-dependent and requires BLM helicase and DNA2 nuclease functions [85]. RPA is essential for both DNA unwinding by BLM and enforcing 5'-3' resection polarity by DNA2. MRN accelerates processing by recruiting BLM to the end. In the other, EXO1 resects the DNA and is stimulated by BLM, MRN, and RPA. BLM increases the affinity of EXO1 for ends, and MRN recruits and enhances the processivity of EXO1 [85].

Metnase possesses a unique endonuclease activity that preferentially acts on ssDNA overhang of a partial duplex DNA [35]. Cell extracts lacking Metnase exhibited significantly lowered end joining activity comparable to those seen in extracts lacking DNA-PKcs or Ku80 [35], whereas cell extracts over-expressing Metnase not only stimulated DNA end joining but also showed an enhanced end processing of non-compatible ends based on DNA sequencing analysis of end joining products [32, 35, 37]. Metnase has no hairpin or loop opening activity [35], indicating that it does not play a role in V(D)J recombination. Given that DNA end processing facilitates end joining by increasing the chance for partial annealing between two non-compatible ends, Metnase's endonuclease activity may play a direct role in stimulating DNA end joining through processing of non-compatible ends. While Metnase contributes to DNA end joining through an enhanced processing of non-compatible ends, its DNA cleavage activity cannot explain Metnase's stimulatory role in the joining of compatible ends [32, 35, 37]. Similar to DNA-PK- and Ku80-defective cells, cell extracts lacking Metnase failed to support joining of compatible ends [32], suggesting that Metnase also has a role in the joining of compatible ends, perhaps by promoting recruitment of the XRCC4-DNA ligase 4 (Lig4) complex [115], an essential player in the ligation step through a physical interaction upon DNA damage. The DNA binding property of Metnase may assist in the localization of DNA Lig4 at the free DNA ends. In this case, Metnase is epistatically above end-processing and subsequent joining, but perhaps below free end recognition and protection, in the NHEJ cascade.

One intriguing thing is how a transposase possesses ssDNA overhang cleavage activity in the absence of TIR sequence. The Metnase-transposase domain has a conserved DDE-like motif (D483, D575, and N610) that is crucial for DNA cleavage activity (Fig. 3) [35, 37, 116]. The function of residues in the DDE-motif includes coordination of a metal ion required for catalysis in other transposases. In addition to these residues, several other residues potentially play a role in the catalytic activity of the transposase domain [116]. Based on the crystal structure of the Metnase-transposase, the active sites of the two subunits that make up

the dimer are distinctly different [116]; one subunit has bound metal in the active site and the other does not [116]. Metal is bound to the active site of one molecule comprising the dimer coordinated to Asp 483 and Asp 575. Residues K445, R578, and H580 within the catalytic pocket adopt different conformations in the metal-bound vs. non-metal bound active site structures and may also play important catalytic functions in ss-overhang cleavage activity. A loop within the active site of Metnase adopts two very different conformations resulting in a translation of a full residue when superimposed such that Arg 578 is located within the active site hydrogen-bonded to Glu 484 in the non-metal bound conformation and flipped out of the active site in the metal bound conformation. Similarly, the position of His 580 is quite different in each of the two different conformations in our structure. Interestingly, each conformation of His 580 is hydrogen-bonded to Glu 484. It remains to be seen what unique feature(s) of the catalytic domain with Metnase is directly linked to its role in DNA repair and replication fork arrest as compared to traditional transposase function.

5. Metnase binding partners in DSB repair

Metnase is a DNA repair factor colocalized with MRN complex and other repair factors at the DNA damage sites [36]. On the other hand, it is a transposase that has a capacity to interact with thousands of potential binding sites (TIR) in human chromosomes [23, 25, 37]. Metnase binds to a specific 19 bp sequence within the consensus Hsmar1 TIR [23, 30, 38, 117]. Similar to other Mariner transposases, the Metnase Helix-Turn-Helix (HTH) motif accounts for this binding; specifically the R432 residue within the HTH region is essential for this binding [37]. In human genomes there are a large number of miniature inverted-repeat transposable elements (MITES). If the solo TIRs are added to the number of MITES, there are approximately 7,000 potential Metnase binding sites in human genome. How does a transposase with a sequence-specific DNA binding activity get localized at the DSB sites? A recent study identified Pso4 as a Metnase binding partner that seems to play a role in Metnase localization at DSB sites [36]. Although Pso4 is Metnase's binding partner, coimmunoprecipitation of Metnase and Pso4 also pulled down the human homolog of Spf27, a member of the Prp19 core complex involved in pre-mRNA splicing [36]. Given that Pso4 is a part of the pre-mRNA splicing complex consisting of Pso4, Cdc5L, Plrg1, and Spf27 [118], the Metnase-Pso4 complex may be a part of the bigger complex including other members of the pre-mRNA splicing complex *in vivo*. Although the physiologic role of the Metnase-Pso4 interaction is still unclear, cells lacking Pso4 failed to show Metnase localization at the DSB sites [36], suggesting that Pso4 play a role in the recruitment of Metnase to the DSB sites. Upon DNA damage, Pso4 is induced [46] and formed a stable complex with Metnase [36]. A recent biochemical analysis suggested several interesting implications for the architecture of the Metnase-Pso4 complex on DNA. First, Metnase dimer forms a 1:1 stoichiometric complex with Pso4 on dsDNA [35, 36]. Although both Metnase and Pso4 can independently interact with TIR DNA, Pso4 is solely responsible for binding to dsDNA once the two proteins form a stable complex [35]. This claim is based on the findings that 1) the Metnase-Pso4 complex interacted with same stoichiometric amount of non-TIR DNA as the TIR DNA, 2)

the Metnase-Pso4 complex interacted with same number of TIR molecules as Metnase or Pso4 alone did, and 3) formation of the Metnase-TIR complex was significantly inhibited by excess of TIR and not by non-TIR, whereas the Metnase-Pso4-TIR complexes were equally inhibited by both TIR and non-TIR DNA [35]. It is possible that Pso4, once forming a complex with Metnase, may directly interfere with Metnase's DNA binding domain (helix-turn-helix motif) [37]. This notion is supported by findings that Metnase bound to TIR DNA went through a conformational change and was less effective than free Metnase in interacting with Pso4 [35]. Pso4 has 6 C-terminal WD-40 repeats [119], a module that is known to interact with post-translationally modified histone 3, including dimethylated-K4 [120]. Given that Metnase HLMT activity targets H3-K4 as well as H3-K36 [32], it is possible that chromatin association of Pso4 may occur via Metnase-mediated H3-K4 methylation, while Metnase requires Pso4 for its DSB localization. Since Pso4 is induced following IR treatment *in vivo* [36, 46, 121], formation of a stable Metnase-Pso4 complex likely occurs in response to DNA damage. The Pso4 also undergoes structural alterations in response to DNA damage [121]. The Metnase-Pso4 complex, once formed, likely goes to nonTIR sites such as DSB sites [36], since Pso4 is solely responsible for binding to DNA in forming the Metnase-Pso4-DNA complex. It would be interesting to see whether Pso4 also affects Metnase's other biochemical functions such as DNA cleavage activity and HLMT activity. Further structural study would be necessary to clarify this intriguing issue.

Metnase also physically interacts with DNA ligase IV (Lig4), an essential DSB repair factor involved in the final end joining step in response to DNA damage [24], which supports the observations that Metnase promoted joining of both compatible and non-compatible ends [32, 35]. It remains to be seen whether Metnase plays a direct role in the recruitment of the XRCC4- Lig4 complex via its interaction with Lig4.

6. Metnase's role in the replication fork arrest

DNA double-strand breaks can be generated at the replication forks when the replication machinery encounters a single-strand break (SSB) or other types of DNA adducts. Attempted replication past a SSB can generate one-sided DSB which topologically differs from DSBs introduced by IR (Fig. 4). One-sided DSB is not a natural substrate for NHEJ, so these breaks can be repaired by homologous recombination repair pathway. Otherwise, it will remain unrepaired generating chromatid breaks, or it may ligate with a DSB in a different chromosome producing radial chromosomes. Stalled replication forks can also regress to generate a chicken-foot structure with a double stranded end (Fig. 4). Such a structure is topologically distinct from IR-induced DSBs in that it encompasses a single double-strand end rather than two double-strand ends.

Metnase possesses a distinct yet undefined role in the replication stress response [122]. Its role appears to be limited to restart of stalled and/or collapsed replication forks. DNA replication analyses indicated that Metnase promotes cell survival only when cells are subjected to replication stress such as hydroxyurea (HU), camptothecin (CT), or UV treatment [122].

Interestingly, when Metnase knockdown cells were treated with HU, the percentage of stopped forks greatly increased and there was a corresponding large decrease in the percentage of continuing forks, while new forks were extremely rare in both HU treated and untreated Metnase knockdown cells [122], indicating that Metnase plays a critical role in restarting stalled replication forks. It also suggests that Metnase may regulate new origin firing when cells experience replication stress. Metnase also regulates the efficiency of replication fork restart, and possibly initiation after replication stress, but it has no effect on the speed of on-going forks [122].

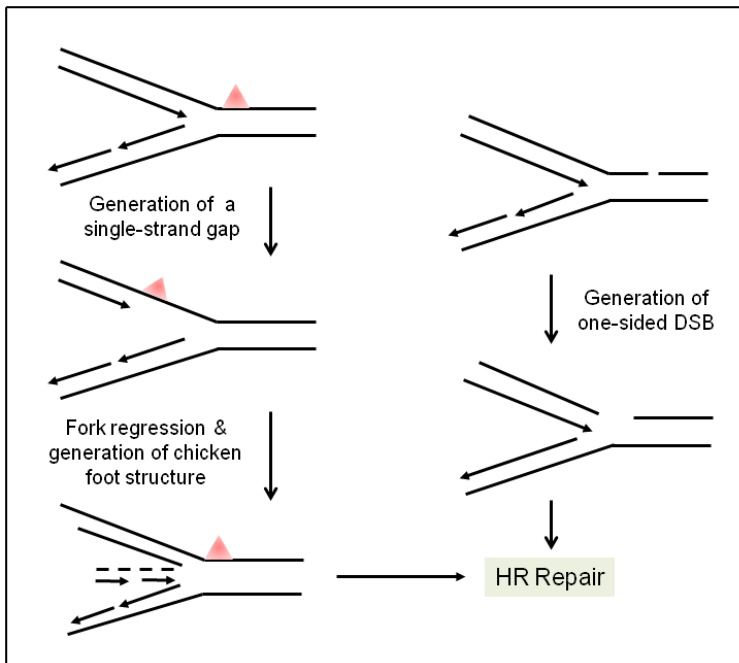


Figure 4. Generation of DSB damage and its repair during replication. Replication forks frequently encounter blocks to their progression including lesions such as single strand breaks. Structures such as a one sided DSB or a chicken-foot structure generated by fork regression can arise as a consequence of such replication stalling and the available evidence suggests a major function of HR is to repair or resolve such lesions.

Interestingly, a recent study with poly ADP-ribose polymerase 1 (PARP-1) revealed that it recruits MRE11 to stalled replication forks [123]. MRE11 with its endonuclease activity may play a role in processing stalled forks leading to RPA recruitment and eventual restart through HR. It is possible that Metnase promotes replication fork restart by promoting NHEJ [124]. NHEJ factors involved in NHEJ are known to promote cell survival after replication stress perhaps by facilitating rejoining of DSEs at collapsed forks [125, 126]. Since each collapsed fork produces only a single broken end that is not a natural substrate for NHEJ, however, it would be highly inaccurate producing radial chromosomes. Another pos-

sibility would be that NHEJ factors promote replication fork restart indirectly through interactions with HR factors [127]. When replication fork stalls, the initial cellular response is to stabilize the replisome to prevent fork collapse. Metnase does not appear to play a role in fork stabilization as similar fractions of cells with collapsed forks were observed regardless of Metnase expression level [122]. Another mechanism by which Metnase could promote fork restart is through its interactions with replisome factors including PCNA and RAD9. Although it is not yet known whether Metnase interacts directly with these proteins, the fact that the Metnase SET domain has a conserved PIP box is highly suggestive of direct interactions. Regardless, our results clearly place Metnase at stalled replication fork. The Metnase SET domain encodes a protein methylase, and Metnase is known to methylate histone H3 and itself [124, 128]. Metnase could regulate PCNA and/or RAD9 function through transmethylation, or it could have a more general effect through chromatin modification. In particular, Metnase targets histone H3 lysines 4 and 36, which are associated with chromatin opening, these modifications could enhance repair factor recruitment to stalled or collapsed forks. Given the well-established role of RAD9 in the intra-S checkpoint response [129], Metnase could promote fork restart by influencing checkpoint activation or downstream checkpoint-dependent processes such as inhibition of origin firing. In addition, Metnase could affect replication fork restart through its direct interaction with Topoisomerase II α (TopoII α). TopoII α is proposed to relax positive supercoils that form ahead of replication forks [130]. Currently there is no information about whether supercoils persist in front of stalled forks. However, when one of the replicative polymerases encounters a blocking lesion, the other polymerase can become uncoupled and progress for a distance, producing a single-stranded gap that is bound by RPA, triggering the intra-S checkpoint [129]. This uncoupled synthesis depends on continued DNA unwinding by the MCM helicase complex, thus positive supercoils will continue to accumulate. By promoting TopoII α -dependent relaxation of these supercoils, Metnase could help create a favorable topological state that assists in fork restart. Conceivably, this could involve restart of stalled forks that are processed to a chicken-foot structure since the resolution of such structures is likely dependent on the topological context of the stalled fork. Alternatively, at collapsed forks, the required HR-mediated invasion of the DSE into the unbroken sister chromatid, require unwinding of the sister duplex and could similarly be affected by the local topological state. Metnase may play different roles depending on the particular state of the stalled or collapsed replication fork.

7. Abnormal expression of Metnase in tumor specimens

The Metnase gene has three exons spread over 13.8 kB located at 3p26, a region of frequent abnormalities in non-Hodgkin's lymphoma, acute and chronic lymphocytic leukemia, myeloma, myelodysplasia, hereditary prostate cancer, and breast cancer (<http://cgap.nci.nih.gov/Chromosomes/Mitelman>). Metnase is expressed in all human tissues tested to various extents (32), with the highest expression in placenta and ovary and the lowest expression in skeletal muscle, which is reminiscent of expression patterns of other DNA repair proteins (131). Interestingly, different transcript variants were found in both normal and cancerous

tissues (23), suggesting that Metnase is broadly expressed and has an important function in human. Metnase is frequently overexpressed in leukemia and breast cancer cell lines, and importantly, downregulating Metnase greatly enhances tumor cell sensitivity to common chemotherapeutics including epididophylotoxins and anthracyclines [132, 133]. Although the precise mechanism(s) by which Metnase promotes restart of the replication fork, Metnase may be a reasonable target for the therapeutic strategies that block DNA synthesis or take advantage of inherent defects of tumor cells in replication fork restart [134, 135].

8. Concluding remarks

While transposase accounts for half of the present organization of the human genome, transposase activity was thought to be extinct in humans probably because unregulated transposition would directly affect genomic stability, resulting in an unacceptably high rate of apoptosis or malignancy [29]. For this reason, transposase functions have been selected against the mammalian organisms [29], which lead to a generation of the SET-Transposase chimeric protein termed Metnase with novel functions in DSB repair, replication fork arrest, and chromosome decatenation that could actually defend the genome against improper DNA movement or DSB damage (Fig. 5).

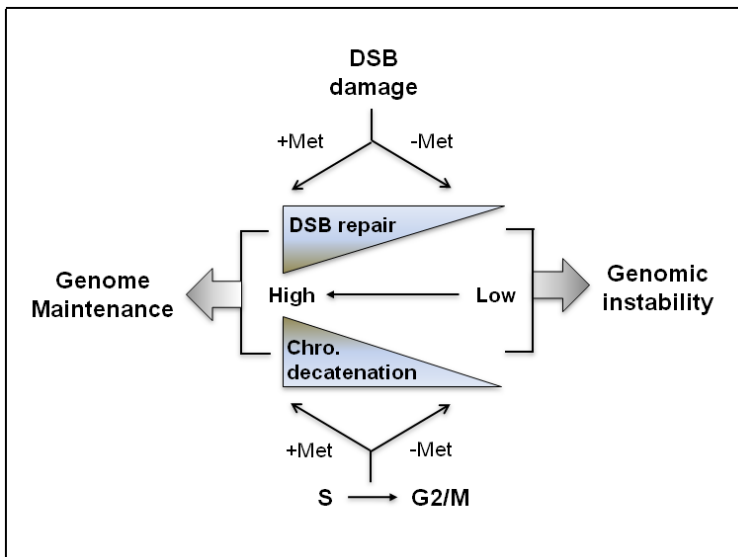


Figure 5. Metnase contributes to genome maintenance by promoting DSB repair and chromosome decatenation.

It should be pointed out that there are no other DNA repair proteins in which the DNA cleavage and histone lysine methyltransferase activities reside within the same protein.

Although the role(s) of Metnase in DSB repair and other DNA metabolism are yet to be defined, a deletion of either the SET or the transposase domain abrogated its function in DNA repair [32], indicating that both domains are essential for this function. Histone lysine methyltransferases (HLMT) is a critical participant in chromatin integrity as evidenced by the number of human diseases including cancers associated with the aberrant expression of its family members [136]. Although the underlying mechanisms of tumorigenesis are still largely unknown, Metnase HLMT targeting of H3K36 dimethylation at DSB damage sites is not only crucial for damage recognition and the early stage of DSB repair, but is also of our interest in tumorigenesis [31]. Metnase may thus be a viable anticancer target for a wide variety of tumor types. Given that altered expression of Metnase affect joining of both compatible- and non-compatible ends [24, 32, 35, 37], Metnase likely have two separate functions in the joining of DSB damage: 1) the Metnase-Lig4 interaction [24] for joining of compatible ends by promoting recruitment of Lig4 complex to DSB sites, and 2) Metnase's structure-specific endonuclease for joining of non-compatible ends by promoting end processing (Fig. 6). Further structure-function studies would be necessary to understand how a transposase becomes an endonuclease with ss-overhang cleavage in a TIR-independent manner.

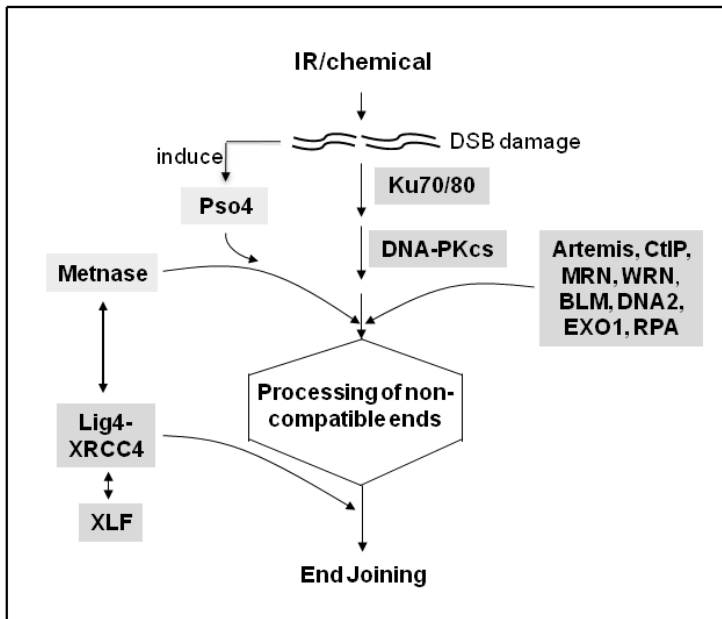


Figure 6. Proposed role(s) for Metnase in DSB repair and chromosome decatenation. Upon DSB damage, the Ku complex first binds to the DNA ends and recruits DNA-PKcs. Metnase binding partner, Pso4 is induced upon DSB damage, which, along with the Ku70/80, likely plays a crucial role in Metnase localization at DSB sites. Metnase's interaction with Lig4 is also induced upon DSB damage, which promotes joining of compatible ends, while Metnase's nuclease activity plays a role in joining of non-compatible ends.

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