Published in final edited form as:

Sci China Life Sci. 2017 October ; 60(10): 1081–1086. doi:10.1007/s11427-017-9164-1.

Regulation of DNA break repair by transcription and RNA

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Abstract

Repair of DNA double-strand breaks (DSBs) via the homologous recombination (HR) pathway is a highly regulated process. A number of proteins that participate in HR are intricately modulated by the cell cycle and chromatin environments of DSBs. Recent studies have revealed a clear impact of transcription on HR in transcribed regions of the genome. Several models have been put forth to explain how the process of transcription and/or its RNA products may influence HR. Here we discuss the results and models from these studies, presenting an emerging view of transcription-coupled DSB repair.

Keywords

DNA break repair; transcription; RNA

Introduction

The influence of transcription on DNA damage repair has been long appreciated. Transcription-coupled nucleotide excision repair (TC-NER) is critical for removal of UV lesions from the template DNA strand of actively transcribed genes, which prevents prolonged stalling of transcription and accumulation of mutations in coding sequences (Hanawalt and Spivak, 2008; Marteijn et al., 2014; Vermeulen and Foustier 2013). Defective TC-NER is linked to the Cockayne Syndrome, a rare but severe genetic disorder characterized by abnormalities including growth failure, premature aging, photosensitivity, and impaired development of nervous system.

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Compliance and ethics The author(s) declare that they have no conflict of interest.

In addition to UV lesions, other types of DNA damage also threaten transcribed and untranscribed regions in the genome. One of the most severe forms of DNA damage is DNA double-strand break (DSB). If not repaired properly, DSBs in transcribed regions give rise to missense, nonsense, frame-shift mutations, as well as deletions and translocations. DSBs are repaired by non-homologous end joining (NHEJ) and homologous recombination (HR) pathways. While NHEJ is error-prone, HR generally has higher fidelity compared to NHEJ. Recent studies have revealed that HR preferentially occurs in transcribed regions of the genome (Aymard et al., 2014; Keskin et al., 2014; Wei et al., 2015), leading to several models in which DSB repair is promoted by the process of transcription, its associated chromatin features, or its RNA products. Here we discuss the possible roles of transcription and RNA in the repair of DSBs.

Evidence of Transcription-Facilitated DSB Repair

HR but not NHEJ proteins are preferentially recruited to DSBs in transcribed regions

To analyze the recruitment of HR and NHEJ proteins to DSBs in different chromosomal environments, Legube and colleagues generated a human cell line (derived from the osteosarcoma cell line U2OS) that inducibly expresses the endonuclease AsiSI (Iacovoni et al., 2010). Induction of AsiSI leads to >100 DSBs throughout the genome in both transcribed and untranscribed regions. Chromatin immuno-precipitation-sequencing (ChIP-Seq) analyses revealed that RAD51, a key HR protein, was enriched at DSBs in actively transcribed regions, but not at those in untranscribed regions (Aymard et al., 2014). In contrast, XRCC4, a factor involved in NHEJ, was similarly recruited to DSBs in both transcribed and untranscribed regions (Aymard et al., 2014). These results suggest that the efficient recruitment of RAD51 to DSBs is associated with active transcription.

Legube and colleagues also used a transcription activator-like nuclease (TALEN) to cleave a specific site within the *GBPI* gene, which is poorly transcribed (Aymard et al., 2014). When *GBPI* transcription was massively induced by IFN- γ treatment, the recruitment of RAD51 to the TALEN cleave site was substantially increased, whereas the recruitment of XRCC4 was not significantly affected. These results demonstrate that active transcription facilitates the recruitment of RAD51 to DSBs.

Independent of the studies by the Legube lab, the Lan lab used the light-excitabile ROS-generating protein KillerRed (KR) to induce DSBs at a Tet-inducible gene (Wei et al., 2015). A number of proteins involved in HR, including RPA, RAD51, RAD51C, and RAD52, were recruited to the DSBs at this locus only when transcription was turned on (Wei et al., 2015). In contrast, the NHEJ protein KU70 was recruited to the DSBs at this locus regardless of the status of transcription. KU70 was excluded from the DSBs in transcribed regions after RPA loading, while retained in untranscribed regions throughout the repair process (Wei et al., 2015). Together, these two complementary studies provided clear evidence that HR but not NHEJ proteins are preferentially recruited to DSBs in actively transcribed regions.

Transcription-coupled DSB repair in yeast

Using *Saccharomyces cerevisiae* as a model organism, Storici and colleagues first showed that synthetic RNA oligonucleotides were able to serve as templates for repair of chromosomal DSBs (Storici et al., 2007). To further investigate whether RNA transcripts contribute to DSB repair, they subsequently developed a reporter assay to follow the repair of an HO endonuclease-induced DSB in the mutated *his3* gene (Keskin et al., 2014). Only when the mutated *his3* gene is corrected by homology-directed DSB repair, wild-type *HIS3* gene is restored and yeast cells are viable in medium lacking histidine. To assess the ability of endogenously generated RNA transcripts to serve as repair templates, *his3* RNA was transcribed from either the HO-targeted locus or a locus away from the HO site. Cleverly, an artificial intron was inserted to the transcribed *his3* gene, so that only the spliced *his3* RNA, but not the unspliced transcript or its DNA template, carries the sequence to restore wild-type *HIS3* gene. Using this assay, Storici and colleagues showed that *his3* RNA generated in cells was able to support *his3* correction (Keskin et al., 2014). Interestingly, when *his3* RNA was generated from the locus away from the HO site, it acted in *trans* to support *his3* correction at the HO site in a manner dependent on the reverse transcriptase SPT3. However, when *his3* RNA was transcribed from the HO-targeted locus, it supported *his3* correction in *cis* independently of SPT3 (Figure 1A), although at low efficiency. These results strongly suggest that DSB repair in transcribed regions could occur in a transcription-coupled manner.

Possible Roles of Transcription in DSB Repair

Transcription-associated chromatin features facilitate HR

Transcription elongation is known to associate with specific histone modifications, one of which is H3K36me3 (Guenther et al., 2007). Legube and colleagues found that knockdown of SETD2, the main H3K36 trimethyltransferase, led to reduced recruitment of RAD51 to DSBs in transcribed regions (Aymard et al., 2014) (Figure 1B). Furthermore, LEDGF, a PWWP domain-containing reader protein of H3K36me3, is also required for the efficient recruitment of RAD51 to DSBs in transcribed regions (Aymard et al., 2014; Eidahl et al., 2013; Pradeepa et al., 2012). Depletion of LEDGF reduced the localization of CtIP, a protein involved in the resection of DNA ends, to DSBs (Daugaard et al., 2012). Thus, transcription-associated H3K36me3 may facilitate HR by promoting resection of DNA ends, which is necessary for the recruitment of RAD51. It is worth noting that a number of chromatin remodeling complexes and histone chaperones are implicated in both transcription and DSB repair. Whether the recruitment of these complexes and proteins to transcribed regions facilitates DSB repair remains to be tested. In addition, it is well known that a subset of histone modifications (e.g., H3K9ac, H3K79me1, H3K4me3) are required for active transcription. How the histone modifications associated with active transcription are altered after damage, and whether they contribute to damage signaling and/or regulate DNA repair, still need further investigations.

CSB may function as a transcription-coupled sensor of DSBs

In search for factors promoting the recruitment of HR proteins to DSBs in transcribed regions, Lan and colleagues tested the possible involvements of the proteins required for TC-

NER (Wei et al., 2015). Knockdown of one of these proteins, CSB (also known as ERCC6), significantly diminished the recruitment of RAD52 and RAD51C to DSBs at an actively transcribed locus. In contrast, knockdown of other NER or TC-NER factors, such as CSA, XPC, and XPG, did not affect the initial recruitment of RAD52 or RAD51C. Furthermore, the interactions of CSB with RAD52 and RAD51C were enhanced after ionizing radiation (IR). The recruitment of RAD52 and RAD51C to DSBs is also dependent on active transcription and the presence of RNA transcripts (Figure 1C). Depletion of CSB resulted in increased IR sensitivity in human cells and embryo developmental aberrations in zebrafish. Interestingly, depletion of CSB not only delayed the repair of DSBs in asynchronously growing cells, but also in cells synchronized in G0/G1, indicating that transcription-coupled DSB repair may occur outside of S/G2 phases of the cell cycle. Another study using CSB knockout (KO) cells generated by rAAV-mediated gene targeting showed that CSB ablation led to a significant reduction in IR-induced nuclear foci of BRCA1, RPA and RAD51, as well as a delayed resolution of IR-induced γ H2AX foci in S/G2 cells (Batenburg et al., 2015). The reduced HR efficiency of CSB KO cells is consistent with their hypersensitive to Olaparib, a PARP inhibitor. Together, these studies established the role of CSB in the transcription-coupled DSB repair.

CSB is known to interact with RNA polymerase II molecules engaged in ternary complexes containing DNA and RNA (Tantin, 1998). During TC-NER, CSB is required for the recruitment of NER factors to sites of UV damage. Similar to its role in TC-NER, CSB is also required for the recruitment of HR factors during the transcription-coupled DSB repair. Whether and how CSB functions in similar or distinctive ways in transcription-coupled NER and DSB repair is not understood. In addition, although TC-NER factors other than CSB (e.g., XPG, CSA, TFIIH) are not required for recruitment of HR proteins to DSBs in transcribed regions, their functions might be important for other aspects of repair or at a later stage. How transcription is coupled with different DNA damage pathways, and how HR is initiated and completed through transcription-dependent and -independent mechanisms, still need further investigation.

Transcription impacts clustering of DSBs

Using the high-throughput sequencing-based chromosome conformation capture (Hi-C) assay, Legube and colleagues studied the clustering of AsiSI-induced DSBs (Aymard et al., 2017). They found that DSBs in actively transcribed regions are more likely to cluster in G1 phase compared to DSBs in transcriptionally inactive regions. Clustering of DSBs requires NBS1, which recognizes DNA ends as part of the MRE11-RAD50-NBS1 (MRN) complex, and the LINC complex, which connects nucleoskeleton and cytoskeleton (Aymard et al., 2017). It is not yet known whether DSB clustering in actively transcribed regions contributes to homology-dependent repair. Notably, the kinetics of DNA repair was similar at clustered and unclustered DSBs in asynchronously growing cells. However, clustered DSBs were repaired more slowly than unclustered DSBs in G1, although both populations of DSBs were efficiently recognized by XRCC4 (Aymard et al., 2017). These results suggest that the NHEJ pathway may be preferentially suppressed by the clustering of DSBs in G1. Thus, it is possible that clustering of DSBs in transcribed regions in G1 prevents the error-prone NHEJ,

which may allow the DSBs to be repaired by the more accurate HR pathway in the following S and G2 phases (Figure 1D).

Possible Roles of Rna In Dsb Repair

The aforementioned studies suggest that the process of transcription and/or its associated chromatin features may affect DSB repair. In the following section, we will discuss how transcription may indirectly influence DSB repair through its RNA products.

DNA:RNA hybrids are required for efficient DSB repair

In yeast, Storici and colleagues showed that the RNA-mediated DSB repair at the mutated *his3* gene was dependent on Rad52 (Keskin et al., 2014). *In vitro*, both purified yeast Rad52 and human RAD52 proteins were able to promote DNA:RNA hybridization (Keskin et al., 2014). RNase H1/2 are enzymes that specifically cleave the RNA in DNA:RNA hybrids. Deletion of *Rnh1* and *Rnh201* genes, which encode the RNase H enzymes in yeast, significantly increased the RNA-mediated DSB repair at the mutated *his3* gene, suggesting that DNA:RNA hybrids contribute to this repair process (Keskin et al., 2014). In human cells, Lan and colleagues found that retention of RAD52 at DSBs in an actively transcribed region was abolished by RNase H treatment (Wei et al., 2015). Together, these two studies suggest that DNA:RNA hybrids are involved in DSB repair in transcribed regions.

The RNA in DNA:RNA hybrids may contribute to DSB repair in several different ways. For example, RNA may function as a template for DNA synthesis at DSBs (discussed below). RNA may hybridize with the ssDNA from two DNA ends, bringing these DNA ends together and promoting ligation of 5' and 3' DNA termini. The RNA anchored to DSBs by DNA:RNA hybrids may function as a scaffold for the assembly of repair complexes. Furthermore, DNA:RNA hybrids coupled with DNA strand breaks might lead to structure distortions, which may be recognized by specific repair proteins as binding substrates. Consistent with the idea that DNA:RNA hybrids promote the recruitment of specific repair proteins, RNase H treatment only abolished the retention of RAD52 but not RAD51C at DSBs in an actively transcribed region (Wei et al., 2015). These possible roles of RNA in DSB repair are not mutually exclusive. Whether and how RNA operates in DSB repair through these mechanisms requires further investigation.

RNA may serve as a repair template

As mentioned above, in yeast the spliced RNA transcript from the *his3* locus can support the homology-directed restoration of wild-type *HIS3* gene (Keskin et al., 2014). Because the mutation in the *his3* gene can only be corrected by using the spliced RNA transcript as a template for DNA synthesis, the study by Storici and colleagues convincingly demonstrates that RNA transcripts are able to serve as templates for DSB repair (Keskin et al., 2014). At least *in vitro*, yeast DNA polymerases α and δ are able to extend DNA primers on RNA-containing templates for a few nucleotides (Storici et al., 2007), raising the possibility that in cells these DNA polymerases may use RNA as a template to extend the 3' ssDNA overhangs of DSBs. The RNA-templated extension of DNA ends may generate complementary sequences between a pair of ssDNA overhangs of a single DSB, allowing

the two DNA ends to capture each other by ssDNA annealing. The ssDNA gaps at these annealed DNA ends may be subsequently filled by DNA polymerases, and the repair process is finally completed by ligation of DNA nicks (Figure 1A). Whether RNA synthesis is used during repair is not yet known. The ribonucleotides incorporated into the repaired DNA strand could be subsequently removed by RNase H2.

In human cells, Lan and colleagues showed that both RAD52 and RAD51C were efficiently recruited to the KR-induced DSBs at an actively transcribed locus even in G0/G1 (Wei et al., 2015). In addition, the KR-induced DSBs were efficiently repaired in G0/G1 cells. Given that HR cannot occur using sister chromatids as a template in G0/G1, the HR factors recruited to transcribed regions may use RNA as a template for DSB repair. It should be noted that the canonical HR pathway is suppressed in G1 (Orthwein et al., 2015). Whether the HR factors recruited to transcribed regions in G0/G1 function through an RNA-templated non-canonical HR pathway remains to be investigated.

Quantitative control of DNA:RNA hybrids during DSB repair

Although DNA:RNA hybrids may promote DSB repair, their proper removal may also be necessary for accurate repair. In *Schizosaccharomyces pombe*, deletion of the genes encoding RNase H led to reduced HR at a DSB generated by the I-Pop I endonuclease, and decreased cell survival after DSB induction (Ohle et al., 2016). Consistent with its role in removing the RNA in DNA:RNA hybrids, depletion of RNase H resulted in increased levels of DNA:RNA hybrids at the I-Pop I site. Interestingly, in cells lacking RNase H, RPA was efficiently recruited to a locus 2 kb from the DSB, but its association with a locus 10 kb from the DSB was reduced compared to that in control cells expressing endogenous RNase H. Conversely, overexpression of RNase H strongly increased the association of RPA with the locus 10 kb from the DSB. These results suggest that extensive resection of DSB is normally inhibited by the accumulation of DNA:RNA hybrids. If levels of DNA:RNA hybrids are too high during DSB repair, DNA ends may not be resected efficiently and the rate of HR may be consequently reduced.

Small RNAs and DSB repair

In addition to the RNA transcripts in actively transcribed regions, small RNAs have also been implicated in the repair of DSBs. In *Arabidopsis*, small RNAs were induced by I-SceI-generated DSBs and detected in the vicinity of these DSBs (Wei et al., 2012). Both sense and antisense small RNAs were detected by deep sequencing. The generation of DSB-induced small RNAs (diRNAs) in *Arabidopsis* requires the plant-specific RNA Polymerase IV and Dicer-like proteins, DCLs. Both DCLs and Ago2, which is bound to diRNAs, are required for efficient DSB repair in *Arabidopsis*.

While the genesis of diRNAs is clear in *Arabidopsis*, this process is still poorly understood in mammalian cells. diRNAs were reported in human cells after induction of I-SceI-generated DSBs (Wei et al., 2012). Knockdown of Dicer and Ago2 reduced HR efficiency in the DR-GFP reporter assay (Wei et al., 2012). Dicer- and Drosha-dependent small RNAs (DDRNs) were also reported in human and mouse cells after DNA damage (Francia et al.,

2012). Depletion of Dicer and Drosha led to defective accumulation of phosphorylated ATM, MDC1, and 53BP1 at DSBs (Francia et al., 2012).

How small RNAs are induced by DSBs in mammalian cells still needs further investigation. diRNAs and DDRNAs were detected in the vicinity of I-SceI-generated DSBs (Francia et al., 2012; Wei et al., 2012). These small RNAs may arise from the transcription start sites near DSBs. Although DSB formation may lead to stalling of RNA Pol II in actively transcribed regions, RNA Pol II may switch to an alternative functional mode to produce divergent small RNAs around the transcription start sites flanking DSBs.

How diRNAs and DDRNAs promote DSB repair in mammalian cells is also elusive. Surprisingly, incubation of Dicer-depleted cells with synthetic small RNAs or small RNAs generated by Dicer *in vitro* suppressed their defects in the DSB response (Francia et al., 2012). This suppression by small RNAs requires the nuclease activity of MRE11, suggesting that DDRNAs promotes DSB repair in a MRE11-dependent manner. While these results support the idea that small RNAs promote DSB repair, they raise the question as to whether DDRNAs function in a sequence-dependent manner. In contrast to the MRE11 model, a more recent study suggested that Ago2 interacts with RAD51 and facilitates the localization of RAD51 to DSBs (Gao et al., 2014). Ago2 interacts with RAD51 independently of its diRNA-binding and catalytic activities, yet these activities of Ago2 are required for RAD51 localization to DSBs. In contrast to both models above, another study suggested that diRNAs promote DSB repair by facilitating the recruitment of MMSET and Tip60 to DSBs (Wang and Goldstein, 2016). How DDRNAs and diRNAs promote DSB repair still requires further investigation.

Perspectives

As we have discussed above, although the involvement of transcription and RNA in DSB repair has become increasingly clear, the molecular mechanisms by which they function remain poorly understood. We still know little about the RNAs that participate in DSB repair. Are these RNAs transcribed before damage or induced by damage? Do they function primarily in *cis* or in *trans*? Are they processed or modified during repair? Interestingly, a recent study showed that a specific RNA modification (m6A) is induced by DNA damage and contributes to the removal of UV induced lesions (Xiang et al., 2017). Whether RNA modifications are involved in DSB repair remains to be tested. In addition to the lack of knowledge on RNAs, we have very limited information of the proteins that function with RNAs. Which proteins bind RNAs during repair? Do RNAs regulate these proteins? Identification of the RNAs and RNA-binding proteins contributing to DSB repair will be an important step toward understanding the mechanisms by which RNAs function in repair. The functional significance of RNA in DSB repair is another important issue to address. Although RNA is able to serve as a repair template in yeast, its efficiency is very low. Is RNA particularly important for DSB repair in specific contexts with respect to local features of DNA, RNA, and histones? If so, what are these contexts? How is DSB repair differentially regulated by RNA in these contexts? Understanding the roles of transcription and RNA in DSB repair under various physiological and pathological conditions will be a major challenge ahead in future studies.

Acknowledgments

This work was supported by NIH grants (GM076388 and CA197779 to Lee Zou, GM118833 to Li Lan).

References

- Aymard F, Aguirrebengoa M, Guillou E, Javierre BM, Bugler B, Arnould C, Rocher V, Iacovoni JS, Biernacka A, Skrzypczak M, Ginalski K, Rowicka M, Fraser P, Legube G. Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes. *Nat Struct Mol Biol.* 2017; 24:353–361. [PubMed: 28263325]
- Aymard F, Bugler B, Schmidt CK, Guillou E, Caron P, Briois S, Iacovoni JS, Daburon V, Miller KM, Jackson SP, Legube G. Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat Struct Mol Biol.* 2014; 21:366–374. [PubMed: 24658350]
- Batenburg NL, Thompson EL, Hendrickson EA, Zhu XD. Cockayne syndrome group B protein regulates DNA double-strand break repair and checkpoint activation. *EMBO J.* 2015; 34:1399–1416. [PubMed: 25820262]
- Daugaard M, Baude A, Fugger K, Povlsen LK, Beck H, Sørensen CS, Petersen NHT, Sørensen PHB, Lukas C, Bartek J, Lukas J, Rohde M, Jaattela M. LEDGF (p75) promotes DNA-end resection and homologous recombination. *Nat Struct Mol Biol.* 2012; 19:803–810. [PubMed: 22773103]
- Eidahl JO, Crowe BL, North JA, McKee CJ, Shkriabai N, Feng L, Plumb M, Graham RL, Gorelick RJ, Hess S, Poirier MG, Foster MP, Kvaratskhelia M. Structural basis for high-affinity binding of LEDGF PWWP to mononucleosomes. *Nucleic Acids Res.* 2013; 41:3924–3936. [PubMed: 23396443]
- Francia S, Michelini F, Saxena A, Tang D, de Hoon M, Anelli V, Mione M, Carninci P, d'Adda di Fagagna F. Site-specific DICER and DROSHA RNA products control the DNA-damage response. *Nature.* 2012; 488:231–235. [PubMed: 22722852]
- Gao M, Wei W, Li MM, Wu YS, Ba Z, Jin KX, Li MM, Liao YQ, Adhikari S, Chong Z, Zhang T, Guo CX, Tang TS, Zhu BT, Xu XZ, Mailand N, Yang YG, Qi Y, Rørdam Danielsen JM. Ago2 facilitates Rad51 recruitment and DNA double-strand break repair by homologous recombination. *Cell Res.* 2014; 24:532–541. [PubMed: 24662483]
- Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell.* 2007; 130:77–88. [PubMed: 17632057]
- Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. *Nat Rev Mol Cell Biol.* 2008; 9:958–970. [PubMed: 19023283]
- Iacovoni JS, Caron P, Lassadi I, Nicolas E, Massip L, Trouche D, Legube G. High-resolution profiling of γ H2AX around DNA double strand breaks in the mammalian genome. *EMBO J.* 2010; 29:1446–1457. [PubMed: 20360682]
- Keskin H, Shen Y, Huang F, Patel M, Yang T, Ashley K, Mazin AV, Storici F. Transcript-RNA-templated DNA recombination and repair. *Nature.* 2014; 515:436–439. [PubMed: 25186730]
- Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JHJ. Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat Rev Mol Cell Biol.* 2014; 15:465–481. [PubMed: 24954209]
- Ohle C, Tesorero R, Schermann G, Dobrev N, Sinning I, Fischer T. Transient RNA-DNA hybrids are required for efficient double-strand break repair. *Cell.* 2016; 167:1001–1013.e7. [PubMed: 27881299]
- Orthwein A, Noordermeer SM, Wilson MD, Landry S, Enchev RI, Sherker A, Munro M, Pinder J, Salsman J, Deltre G, Xia B, Peter M, Durocher D. A mechanism for the suppression of homologous recombination in GI cells. *Nature.* 2015; 528:422–426. [PubMed: 26649820]
- Pradeepa MM, Sutherland HG, Ule J, Grimes GR, Bickmore WA. Psip1/Ledgf p52 binds methylated histone H3K36 and splicing factors and contributes to the regulation of alternative splicing. *PLoS Genet.* 2012; 8:e1002717.
- Storici F, Bebenek K, Kunkel TA, Gordenin DA, Resnick MA. RNA-templated DNA repair. *Nature.* 2007; 447:338–341. [PubMed: 17429354]

- Tantin D. RNA polymerase II elongation complexes containing the Cockayne syndrome group B protein interact with a molecular complex containing the transcription factor IIIH components xeroderma pigmentosum B and p62. *J Biol Chem.* 1998; 273:27794–27799. [PubMed: 9774388]
- Vermeulen W, Fousteri M. Mammalian transcription-coupled excision repair. *Cold Spring Harb Perspect Biol.* 2013; 5:a012625. [PubMed: 23906714]
- Wang Q, Goldstein M. Small RNAs recruit chromatin-modifying enzymes MMSET and Tip60 to reconfigure damaged DNA upon double-strand break and facilitate repair. *Cancer Res.* 2016; 76:1904–1915. [PubMed: 26822153]
- Wei L, Nakajima S, Bohm S, Bernstein KA, Shen Z, Tsang M, Levine AS, Lan L. DNA damage during the G0/G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination. *Proc Natl Acad Sci USA.* 2015; 112:E3495–E3504. [PubMed: 26100862]
- Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, White CI, Rendtlew Danielsen JM, Yang YG, Qi Y. A role for small RNAs in DNA double-strand break repair. *Cell.* 2012; 149:101–112. [PubMed: 22445173]
- Xiang Y, Laurent B, Hsu CH, Nachtergaele S, Lu Z, Sheng W, Xu C, Chen H, Ouyang J, Wang S, Ling D, Hsu PH, Zou L, Jambhekar A, He C, Shi Y. RNA m6A methylation regulates the ultraviolet-induced DNA damage response. *Nature.* 2017; 543:573–576. [PubMed: 28297716]

Figure 1.

Possible roles of transcription and RNA in DSB repair. A, RNA may serve as a bridge between two DNA ends, or a template to extend the 3' DNA end. RNA is labeled in green. The DNA generated by RNA-templated DNA synthesis is labeled in red. B, Transcription-associated H3K36me3 may recruit LEDGF and promote CtIP mediated DSB resection. C, CSB may function in an RNA Pol II-coupled manner to sense DSBs in transcribed regions and recruit HR proteins. D, Transcription may promote clustering of DSBs in G1 and prevent error-prone NHEJ, which would indirectly promote HR at the DSBs in the following S/G2 phases.