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DNA repair by RNA: Templated, or not templated, that is the question

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Abstract

Cells are continuously exposed to both endogenous and exogenous sources of genomic stress. To maintain chromosome stability, a variety of mechanisms have evolved to cope with the multitude of genetic abnormalities that can arise over the life of a cell. Still, failures to repair these lesions are the driving force of cancers and other degenerative disorders. DNA double-strand breaks (DSBs) are among the most toxic genetic lesions, inhibiting cell ability to replicate, and are sites of mutations and chromosomal rearrangements. DSB repair is known to proceed via two major mechanisms: homologous recombination (HR) and non-homologous end joining (NHEJ). HR reliance on the exchange of genetic information between two identical or nearly identical DNA molecules offers increased accuracy. While the preferred substrate for HR in mitotic cells is the sister chromatid, this is limited to the S and G2 phases of the cell cycle. However, abundant amounts of homologous genetic substrate may exist throughout the cell cycle in the form of RNA. Considered an uncommon occurrence, the direct transfer of information from RNA to DNA is thought to be limited to special circumstances. Studies have shown that RNA molecules reverse transcribed into cDNA can be incorporated into DNA at DSB sites via a non-templated mechanism by NHEJ or a templated mechanism by HR. In addition, synthetic RNA molecules can directly template the repair of DSBs in yeast and human cells via an HR mechanism. New work suggests that even endogenous transcript RNA can serve as a homologous template to repair a DSB in chromosomal DNA. In this perspective, we will review and discuss the recent advancements in DSB repair by RNA via non-templated and templated mechanisms. We will provide current findings, models and future challenges investigating RNA and its role in DSB repair.

Keywords

RNA-templated DNA repair; Central dogma; cDNA; Double-strand break repair; RNase H; Homologous recombination; NHEJ; Rad52; Reverse transcription; LINE-1; Retrotransposon

1. Surviving the tragedy of DNA breaks

The accurate transfer of genetic information is an essential requirement that has allowed life to precisely transmit information from one generation to the next. Over the billions of years

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Conflict of interest

The authors declare no conflict of interest.

DNA based life has existed on Earth, the transfer of genetic materials has continuously been bombarded with exogenous and endogenous threats including ionizing radiation, replication stress, and reactive oxygen and nitrogen species. These threats result in a diverse set of genetic abnormalities including but not limited to modified bases, mispaired bases, cross-linkage, nicked DNA and double-stranded breaks (DSBs). In particular, DSBs in DNA are one of the most severe events a cell can experience. DSBs are hazards to genome integrity, causing mutations, chromosomal rearrangements or cell death if not precisely repaired [1]. To contend with these threats, cells evolved two major repair mechanisms for DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is an error prone process of reattachment of broken DNA ends, favored in the G0/G1 phase of the cell cycle [2]. In contrast, HR involves the exchange of genetic information between homologous or homeologous DNA sequences and is thus generally active in the S and G2 phases of the cell cycle, allowing for accurate repair by a sister chromatid in mitotic cells [3]. Homologous DNA substrates that facilitate repair are in relative short supply when compared to the abundance of homologous substrate that may be present in the form of transcript RNA. However, the transfer of genetic information from RNA to DNA is considered a rare phenomenon. Could RNA play a significant role in DSB repair?

2. Central dogma reversed

In the central dogma of molecular biology, the general transfers of genetic information: DNA $\rightarrow$ DNA, DNA $\rightarrow$ RNA, and RNA $\rightarrow$ protein are believed to occur in most cells; while the special transfers: RNA $\rightarrow$ RNA, RNA $\rightarrow$ DNA, and DNA $\rightarrow$ protein are restricted to specific sequences, or have only been shown *in vitro* [4,5]. The transfer of RNA $\rightarrow$ DNA, reverse transcription, is known to happen in retroviruses, retrotransposons, and during telomere synthesis [6,7]. Remarkably, there is mounting evidence showing that the transfer of RNA $\rightarrow$ DNA is not merely restricted to these special cases, but is a more general phenomenon in nature. The first evidence for an RNA $\rightarrow$ DNA transfer of genetic information not limited to nucleic acid of retroelements came from experiments in the early 1990s on the reverse transcriptase (RT) activity of the yeast retrotransposons (Tys). These studies showed that: i) not only is RNA originating from retroelements reverse transcribed in yeast, but potentially any cellular RNA, such as the RNA deriving from the yeast HIS3 marker gene, may be substrates of reverse transcription, and that ii) RNA reverse transcribed into a DNA copy (cDNA) can modify genomic DNA [8–12]. The cDNA products are potential substrates for recombination between homologous or homeologous sequences or can be captured at sites of a DSB [13–15]. The work of others on the function of the mammalian retrotransposon Long Intersperse Element-1 (LINE-1) found the capture of endogenous mRNA sequences, in addition to LINE-1 mRNA, at sites of DNA damage [8,16]. Overall these findings indicate that the transfer of RNA $\rightarrow$ DNA is not solely limited to special cases and sequences. As discussed below, more recent experimental data have continued to provide support for an RNA $\rightarrow$ DNA transfer of genetic information from ‘generic’ RNA sequences, not deriving from retroelements or telomeres, even without a cDNA intermediate. Therefore, we propose a broader model in which the transfer of RNA $\rightarrow$ DNA is also a general type of genetic transfer in the process of RNA-mediated DNA repair (Fig. 1).

3. RNA on the frontlines of DNA damage

3.1. RNA-mediated, non-templated DSB repair

The study of the retrotransposon activity in yeast offered the first glimpse into the effects of RNA in the repair of DNA. Using an HO-endonuclease induced DSB at the mating type locus (*MAT*) in yeast lacking both *HML* and *HMR* donor templates that normally repair *MAT*, Moore and colleagues showed the capture of Ty sequences at the site of the HO DSB. The capture of short ~100 bp segments appeared independent of the HR protein Rad52 [14]. Additionally, a *his3* marker gene with an artificial intron inserted into the antisense orientation relative to *HIS3* gene was fused to an inducible Ty1 element on a plasmid and upon activation of Ty1 transcription and DSB induction, fused *Ty1/HIS3* cDNA was found integrated at the site of an induced DSB [15]. Additional studies also found Ty1 cDNA inserted at sites of DSBs but appeared dependent upon NHEJ factors [17]. Building off of these experiments, we illustrate a model of RNA-mediated, non-templated cDNA insertion at sites of a DSB, in which RT activity of Ty elements generates cDNA molecules, that are then hijacked by NHEJ machinery and inserted at sites of DSBs (Fig. 2(A)). Ty integrase can also facilitate the integration of non-Ty1 DNA fragments similar to cDNA containing the conserved terminal dinucleotide 5'-TG-CA-3' [18].

3.2. Non-LTR retrotransposon mediated DSB primed reverse transcription

The model for LINE-1 retrotransposition is built from studies of the R2 element in silkworm *Bombyx mori*. It occurs via a target-primed reverse transcription mediated mechanism following endonuclease cleavage [19]. In this model of human LINE-1 retrotransposition, endonuclease activity of the LINE-1 element cleaves DNA, generating a 3'-OH end that is used as a primer for reverse transcription; a second cleavage on the adjacent strand primes the second round of cDNA synthesis and integration of the LINE-1 element into the genome [20]. Studying an endonuclease deficient LINE-1 element in NHEJ-defective Chinese hamster ovary cells, Morrish et al. found that LINE-1 retains the ability to retrotranspose, leading to the proposal that LINE-1 elements can integrate at sites of DNA lesions [16,21]. In support of this model, computational screens identified L1 loci lacking characteristic structural features of target-site primed reverse transcription suggesting an endonuclease-independent L1 insertion at DNA DSBs, providing a role of L1 elements in DSB repair [22]. LINE-1 elements not only transpose themselves but also other retroelements or cellular RNA [8]. In response to DNA damage, LINE-1 reverse transcriptase can use free DNA ends as a primer for the insertion and repair of DSBs (Fig. 2(B)). Could cellular RNA be used in the repair of their own genes with the aid of L1 reverse transcriptase? As described below from experiments in budding yeast, not only the cDNA reverse transcribed from the RNA transcript but also the RNA transcript itself can serve as template for repair of a break in the same DNA gene that generated the transcript RNA. Such RNA-driven DNA repair mechanism may work even more efficiently in mammalian than in yeast cells. In fact, differently from Ty reverse transcription that occurs inside Ty particles in the cytoplasm of yeast cells [23], LINE-1 reverse transcription occurs in the nucleus and can be primed by genomic DNA sequences to which the transcript anneals [16]. Therefore, LINE-1 RT may efficiently synthesize DNA from a transcript template annealed to the broken DNA ends of a mammalian DNA sequence, and thus facilitate DNA repair.

3.3. Homologous recombination with cDNA intermediates

cDNA molecules are short dsDNA intermediates of retrotransposition. If cDNA molecules are homologous to genomic regions, could they be used by the HR machinery? Indeed, Ty cDNA of yeast can recombine in a HR manner with homologous Ty elements encoded in the genome, and this process is partially dependent on *RAD52* [24,25]. DSBs inside of Ty elements can increase recombination between these elements and possibly cDNA intermediates [26]. The ability of Ty elements to generate cDNA molecules of not only Ty RNA but also endogenous transcripts is a proposed model to explain the paucity of introns in the *Saccharomyces cerevisiae* genome and the 5'-bias in genes that retain them [27]. To test this, Derr and colleagues developed an assay using a *HIS3* reporter gene interrupted by an artificial intron inserted in the antisense orientation. The gene cassette was integrated into an episomal vector under the control of an inducible promoter (galactose inducible promoter, *pGAL1*) in the antisense orientation relative to the native *HIS3* promoter in the sense orientation. Upon galactose induction, transcription and splicing generated an antisense *his3* mRNA which, if reverse transcribed by Ty RT, could be integrated into the genome resulting in His⁺ prototrophic cells. The genome contained a deletion of *his3*, which resulted in His⁻ auxotrophic cells with no homology to *his3* located on the plasmid. Upon galactose induction of the artificial intron containing the antisense *his3* gene cassette, His⁺ prototrophic cells were observed at a frequency of around 200 per 10⁹, half due to integration into the chromosome and half to integration as a result of *his3* cDNA HR with *his3* on the plasmid. The reverse transcription of endogenous cellular RNA and its integration into the genome raises the possibility of recombination events initiated directly by RNA molecules [9]. Using a similar system, in which, a *his3* reporter marker under the regulation of galactose inducible promoter and containing an artificial intron in the antisense orientation was inserted in the yeast genome, Keskin et al. detected repair of an induced DSB in *his3* by *his3* cDNA via HR. If a cDNA molecule has homology with regions around a DSB, the cDNA can be used as a substrate for repair of that DSBs (Fig. 2C). Furthermore, inactivation of ribonuclease (RNase) H function by deletion of the RNase H1 gene (*RNH1*) and the catalytic subunit of RNase H2 (*RNH201*) significantly increased the frequency of cDNA recombination, likely through the presence of cDNA-RNA hybrids [10–12]. *In vitro* and *in vivo* data support the finding that RNase H1 can inhibit reverse transcriptase processivity [28]. However, *in vivo* data suggest that RNase H2 is the preferred RNase H enzyme targeting cDNA in yeast [12]. It is of interest that defects in RNase H2 associated with the inflammatory Aicardi-Goutières syndrome (AGS) lead to an increase in RNA-DNA hybrids [29]. The yeast AGS orthologous mutations of RNase H2 when combined with RNase H1 null mutation result in elevated cDNA-driven repair of a DSB [12]. This hints at a rise in the levels of RNA-DNA hybrids and cDNA in AGS patients that are substrates for HR machinery and genome instability. Furthermore, the absence of RNase H activity results in increased RNA-DNA hybrids associated with retrotransposons [30]. It is interesting to speculate if mutants of factors that bind free dsDNA, like those of NHEJ machinery and DSB resection enzymes, could have any effect on cDNA mediate recombination, allowing for increased stability and access to the recombination hardware [31,32].

3.4. Direct RNA-templated DNA repair

As HR is thought to be limited to DNA repair of DSBs in the S/G2 phase of the cell cycle when a sister chromatid is available, a mechanism for accurate repair of a DSB in G0/G1 appears lacking. It was hypothesized that RNA transcripts could provide an alternative template for accurate repair [33]. Studies by Xu et al. also proposed the involvement of RNA templated HR to explain the high frequency of homozygosity in rice [34]. To explore the possibility of a direct RNA-templated DNA repair by HR, RNA-only or RNA/DNA chimeric single-stranded oligonucleotides were transformed into yeast cells and used to repair a broken *leu2* marker locus in yeast chromosomal DNA leading to Leu⁺ transformants. Deletion of the *SPT3* gene, essential for Ty1 and Ty2 transposition, had no effect on the repair frequency at the *leu2* locus, signifying the ability of RNA-containing oligonucleotides to directly template the repair of a DSB in yeast cells [35]. In addition to yeast, RNA-containing and RNA-only oligonucleotides could also template DSB repair in homologous chromosomal DNA containing the green fluorescent protein (GFP) gene after DSB induction in human embryonic kidney cells [36]. Building off the work that synthetic RNA oligonucleotides can facilitate the repair of DSBs, Keskin et al. showed not only cDNA serving as a template for repair of a DSB through HR but also the direct repair by transcript-RNA in the absence of RNase H function [11]. Transcript-RNA-templated DSB repair appears to be strongly influenced by the proximity of the transcript relative to the site of the DSB. RNA deriving from the same locus (*cis*) as the break has a greater repair frequency opposed to RNA deriving from distant parts of the genome (*trans*) to repair a DSB in a homologous locus [11]. These results demonstrate that actively transcribed DNA experiencing a DSB can repair itself using the genetic information on the transcript RNA. These data also show that the transfer of genetic information from RNA to DNA occurs with an endogenous generic transcript, and is thus a more general phenomenon than anticipated.

Direct RNA-templated DNA repair was revealed in the absence of the genes coding for RNase H [11]. However, direct RNA-templated DNA repair may also be active in the presence of wild-type RNase H function. We know that in wild-type cells DSB repair by transcript RNA is initiated efficiently, but it is quickly suppressed by RNase H1 and H2 activity [11]. Defects in RNases H specifically stimulate DSB repair via HR by RNA-containing substrates in the *cis* system, but do not stimulate DSB repair via HR by homologous DNA donor sequence, or via NHEJ [10,11]. Thus, it is possible that the inability to detect DSB repair by transcript RNA in wild-type RNase H cells is not due to lack of such a repair mechanism, but rather to limitations of the experimental system adopted. Experiments using synthetic RNA-containing oligonucleotides showed that RNA sequences could directly template DSB repair in yeast chromosomal DNA in wild-type, *spt3* and *rnh1 rnh201* cells with similar frequency [35]. Thus, a system could be developed to allow for a faster DSB repair by RNA, to avoid attack of the template RNA strand by RNase H before completion of repair.

In vivo and *In vitro* experiments suggest that the Rad52 protein can mediate annealing of complementary RNA and DNA strands; however, DSB repair by transcript RNA was not completely abolished in Rad52-null mutants suggesting a Rad52 independent pathway. There are numerous studies which support HR between RNA and DNA molecules. The

recombinase Rad51 and its *Escherichia coli* ortholog RecA can promote RNA-DNA hybridization, strand exchange *In vitro*, and R-loop formation *in vivo* [37–40]. It was found that Rad51 is preferentially recruited to sites of transcriptionally active chromatin experiencing DSBs [41]. More recently, it was suggested that the Cockayne syndrome protein B (CSB) of transcription coupled nucleotide excision repair pathway exhibits the ability to recruit HR factors including RPA1, RAD51C, RAD51 and RAD52 to DSBs sites being actively transcribed in G0/G1. Furthermore, this recruitment is dependent on the presence of transcript RNA [42]. Not only are DSB repair proteins recruited to sites of DSBs in actively transcribed genes, but actively transcribed genes are repaired at a faster rate [43]. Following these recent findings, it is conceivable that transcript RNA may be playing a role in DSB repair at actively transcribed genes. This suggests that transcript RNA can pair with homologous DNA in *cis* preferentially, but also in *trans* at the site of a DSB and facilitate precise end ligation of the DSB ends or extend the 3' end of the DSB by a yet unknown polymerase (Fig. 2(D) and (E)).

We propose a model in which, the transmission of information from RNA to DNA is a more general transfer than commonly accepted and may have a substantial role in genome stability. We highlight the mechanisms of RNA-driven DNA modification including cDNA-mediated recombination and integration, and direct RNA-templated DNA repair and insertion. Given that RNA molecules are complementary copies of the DNA from which they are derived, it is not an easy task to demonstrate a direct role of RNA in DNA repair. It is likely that we still underestimate the capacity of RNA to mediate non-templated and especially templated modifications into DNA. Thus, new and innovative assays are required to uncover the true roles of RNA in the repair of DNA damage.

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Abbreviations

DSB	double-strand break
HR	homologous recombination
NHEJ	non-homologous end joining
cDNA	complementary DNA
RT	reverse transcriptase
Ty	transposon-yeast
LINE-1	Long Intersperse Element-1
dsDNA	double-stranded DNA
AGS	Aicardi-Goutières syndrome

GFP green fluorescent protein

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Fig. 1. Elaboration of the general transfers of genetic information in the central dogma of molecular biology

DNA is shown in dark blue, RNA in red and protein in turquoise blue. Forward, straight, black arrows represent the direction of the general forms of genetic transfer that occur in cells: DNA $\rightarrow$ RNA and RNA $\rightarrow$ protein. The curved, black arrow indicates the general transfer of DNA $\rightarrow$ DNA occurring in DNA replication and DNA-mediated DNA. The reverse, straight, red arrow marks the proposed more general transfer of RNA $\rightarrow$ DNA occurring during RNA-mediated DNA repair.

Fig. 2. Models of RNA-mediated, non-templated and templated DSB repair

DNA strands are shown in dark blue, RNA in red, and cDNA strands in light blue. DSB induction is pictured by an orange lightning bolt. (A) RNA-mediated, non-templated DSB repair. (B) RNA-templated DSB repair by cDNA insertion. The reverse transcriptase (RT) is represented in blue oval shape. Dotted light blue arrow represents DNA synthesis by the RT. (C) RNA-templated DSB repair by homologous cDNA. The small black line indicates annealing between the DSB end and cDNA. Dotted blue-gray arrow represents DNA synthesis on the template cDNA. (D) RNA-templated DSB repair in *cis* and (E) in *trans*. Rose oval shape represents RNA polymerase. The small black line indicates annealing between the DSB end and the RNA molecule. RNA can bridge the broken DNA ends (left side), or the 3'-DNA end can be extended using RNA as a template (right side). Dotted blue-gray arrow represents DNA synthesis from RNA.