

Repair by retrotransposition

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Published online: 13 May 2002, DOI: 10.1038/ng897

LINE-1 elements, an abundant class of mammalian transposable elements, can insert at sites of double-stranded DNA breaks. Do they promote the repair process or take advantage of it?

Retrotransposons are common components of all eukaryotic genomes. They generate duplicate copies of themselves, and sometimes other genes, by reverse transcribing RNA transcripts. With over 40% of our DNA directly attributed to these retrotransposition events¹, the human genome provides ample evidence for the abundance of these sequences.

Retrotransposons are typically regarded as selfish elements that, like parasites, are adapted to insure their own survival even at the expense of the host. Paradoxically, the progeny of retrotransposons seldom leave the host, which suggests that their survival is tied closely to that of the host. This long term co-existence provides opportunities for joint ventures, possible examples of which include X-inactivation in mammals, telomere maintenance in fruit flies and introduction of new genes or patterns of gene expression in many organisms^{1–3}. In the accompanying paper, Tammy Morrish and colleagues⁴ show that the major retrotransposon of the human genome, the LINE-1 element, may have a role in yet another critical cellular pathway: the repair of double-stranded DNA breaks (DSBs).

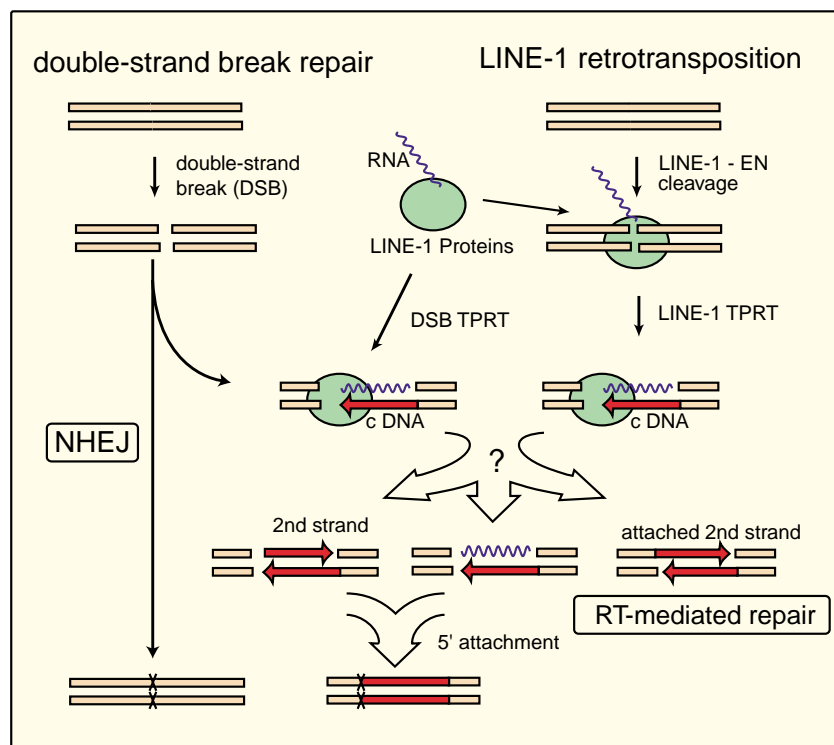
LINE-1 insertions at DSBs

LINE-1 elements are members of the non-long terminal repeat (non-LTR) class of retrotransposons (also called retrotransposons). Although hundreds of thousands of LINE-1 copies exist in mammalian genomes, only a small fraction are active⁵. The critical step in the integration of non-LTR retrotransposons is cleavage of a chromosomal target site by an endonuclease encoded by the LINE-1 element, followed by synthesis of the new DNA copy of the element directly onto the chromosome. This process has been termed target-primed reverse transcription (TPRT). Although the specific steps of TPRT have been elucidated for only one type of non-LTR retrotransposon⁶, Moran and colleagues⁷ previously established a cultured cell assay system that generates LINE-1 integrations with all the properties expected of a TPRT reaction. In this assay,

an artificial intron is inserted within the LINE-1 element in the antisense orientation relative to a selectable marker gene. Integrations brought about by DNA recombination are excluded because the only means of obtaining a functional marker gene is through reverse transcription of a spliced mRNA transcript.

In human cultured cells (HeLa), LINE-1 integrations require both endonuclease and reverse transcriptase activity to be supplied by the marked LINE-1 element⁷. It was thus surprising that when Morrish

*et al.*⁴ conducted the same assay in Chinese hamster ovary cells, low levels of LINE-1 integration could be detected despite the presence of a mutant endonuclease (EN⁻). To test the hypothesis that these LINE-1 integrations occurred at sites of existing DNA breaks in these cells, the authors used cultured cells that were deficient in DSB repair. Cells with deletions or mutations in genes for non-homologous end joining (NHEJ) promoted high levels of LINE-1 integration in the absence of the LINE-1 endonu-



Possible role of LINE-1 retrotransposition in double-strand break (DSB) repair. DSBs are shown being repaired by non-homologous end joining (NHEJ) or entering the LINE-1 retrotransposition pathway. LINE-1 element-encoded proteins (green sphere), including endonuclease and reverse transcriptase activities, bind the LINE-1 RNA transcript (blue wavy line). This RNA-protein complex can either generate its own break in the chromosome or associate with a pre-existing DSB. In both cases, the LINE-1 reverse transcriptase uses the 3' end of the DNA break to prime cDNA synthesis (red arrow) near the 3' end of the LINE-1 transcript, a process termed target-primed reverse transcription (TPRT). After TPRT, it is not known whether the LINE-1 machinery can displace the RNA and synthesize the second DNA strand (second red arrow). One possibility is that LINE-1 uses the other end of the DSB to prime second-strand synthesis. In this case, a reverse transcriptase (RT)-mediated repair has occurred. If the second DNA strand is not made by the LINE-1 machinery or is primed by a means other than the DSB, then the 5' attachment of the LINE-1 sequence will require components of the cellular DNA repair machinery, possibly even NHEJ. Mutations resulting from repair are indicated with an X.

lease activity, whereas cells deficient in homologous recombination did not.

To test whether LINE-1 integration in the NHEJ mutant cells occurred at DSBs, a number of the insertion events were cloned and sequenced. Compared to integrations of fully functional LINE-1 elements, those of EN- elements were unusual in several ways: the EN- insertions were not at sites of normal LINE-1-endonuclease cleavage⁸; target site duplications typically associated with LINE-1 integration had not occurred; reverse transcription had not always initiated within the poly(A) tail at the 3' end of the LINE-1 RNA; and, finally, short sequences derived from other cellular RNAs were occasionally integrated at the 3' end. These properties strongly support a model of LINE-1 integration at DSBs.

Friend or foe?

Do these retrotransposition events aid in the cellular DNA repair process or does the LINE-1 machinery simply take advantage of potential priming sites offered by such breaks without contributing to repair? As outlined in the figure, the answer to this question remains uncertain because little

is known about LINE-1 integration steps subsequent to the initial TPRT. The most satisfying model suggests that the LINE-1 reverse transcriptase uses the 3' end of the DNA at the other end of the DNA break to prime synthesis of the second DNA strand. Such a mechanism would allow the LINE-1 machinery to repair a DSB by a reverse transcriptase-mediated reaction.

Unfortunately, there is no direct evidence as to how, or even whether, the second DNA strand is synthesized by the LINE-1 machinery. Alternative mechanisms to attach the 5' end of LINE-1 elements to the chromosome have been suggested⁵⁻⁷, two of which are shown in the figure. It is quite possible that even in the normal LINE-1 retrotransposition reaction, considerable assistance from the DNA repair machinery—even NHEJ itself—is needed to attach the 5' end of LINE-1 to the chromosome.

Even if LINE-1 elements could participate in reverse transcriptase-mediated repair, would the cell benefit from this process? Reverse transcriptase-mediated repair introduces errors, as each event generates a new insertion. But the process of NHEJ is also extremely error-prone⁹, and mammals seem quite resilient when it

comes to new insertions. Failure to repair DSBs, on the other hand, has been correlated with lethality, aneuploidy and tumorigenesis¹⁰. As such, any means to repair a DSB might be better than the break itself.

It is clear that more work is needed to understand the mechanism of LINE-1 retrotransposition, its use of DSBs, and the involvement of the DNA repair mechanisms. It is remarkable that, despite our detailed understanding of mobile element insertions in bacteria, yeast and insects¹¹, we know so little of the insertional mechanism that accounts for nearly half of our own genome. □

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