R-loops role in RNA-templated DNA repair

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Introduction

In the classical dogma of molecular biology, the general flow of genetic information is believed to be DNA $\rightarrow$ RNA and RNA $\rightarrow$ protein. In recent years, it has become evident that the transfer of information from RNA to DNA can occur as well. One of the most dangerous damages to DNA are double-strand breaks (DSBs), wherein both DNA strands are sliced. The Storici Lab showed that antisense transcript RNA can serve as a template for DSB repair in yeast [1]. It is unclear how the repairs occur, but experimental data indicates that they occur during transcription.

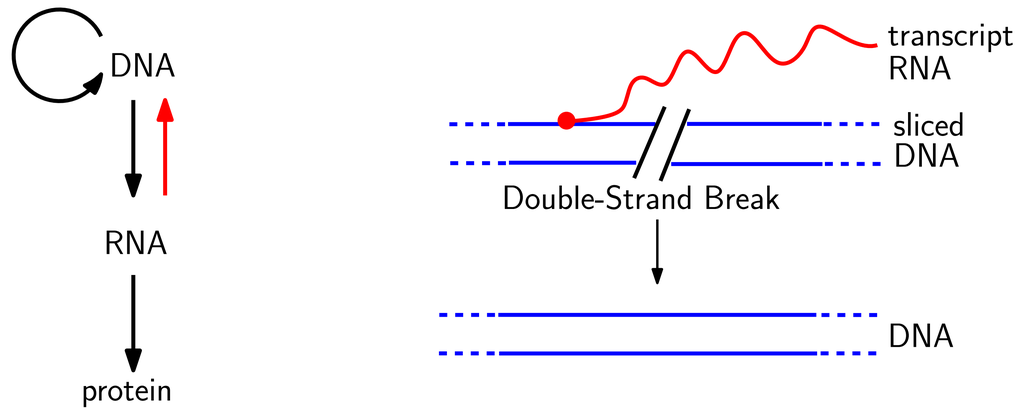

Figure 1: DNA-RNA interaction and double-strand breaks.

Recently, Yusuhara et al. proved that the presence of R-loops nearby DSBs promotes precise repair of the DSBs in human cells [2]. R-loops are three-stranded structures formed when the transcript RNA re-anneals with its template strand during transcription.

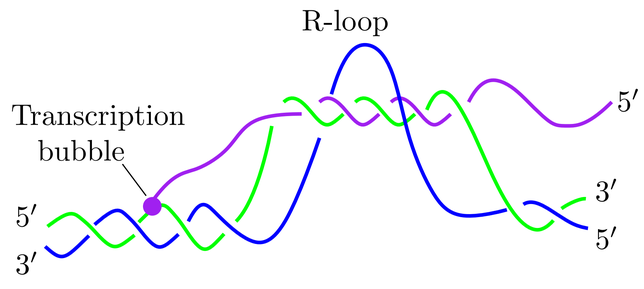

Figure 2: Schematic representation of an R-loop.

In [2], the authors do not examine whether RNA is the template for DSB repair. However, their results suggest a mechanism for RNA-templated DSB repair in human cells, where the antisense transcript RNA engages with the ssDNA of the R-loop formed by the sense transcript, and repairs the DNA.

Experimental Design

We propose to induce one DSB by using CRISPER-CAS9 system in GAPDH gene (a transcriptionally active gene) in a location closed to a DNA region which is prone for R-loop formation. The cells can be transfected with in-vitro transcribed antisense RNA obtained by transcribing the DsRed red fluorescent marker gene. RNA can be engineered to have homology with the GAPDH gene around the DSB location, including the R-loop. DSB repair by the in-vitro transcribed antisense RNA can repair the damaged DNA by inserting the DsRed sequence into the GAPDH gene, thus generating red fluorescent cells.

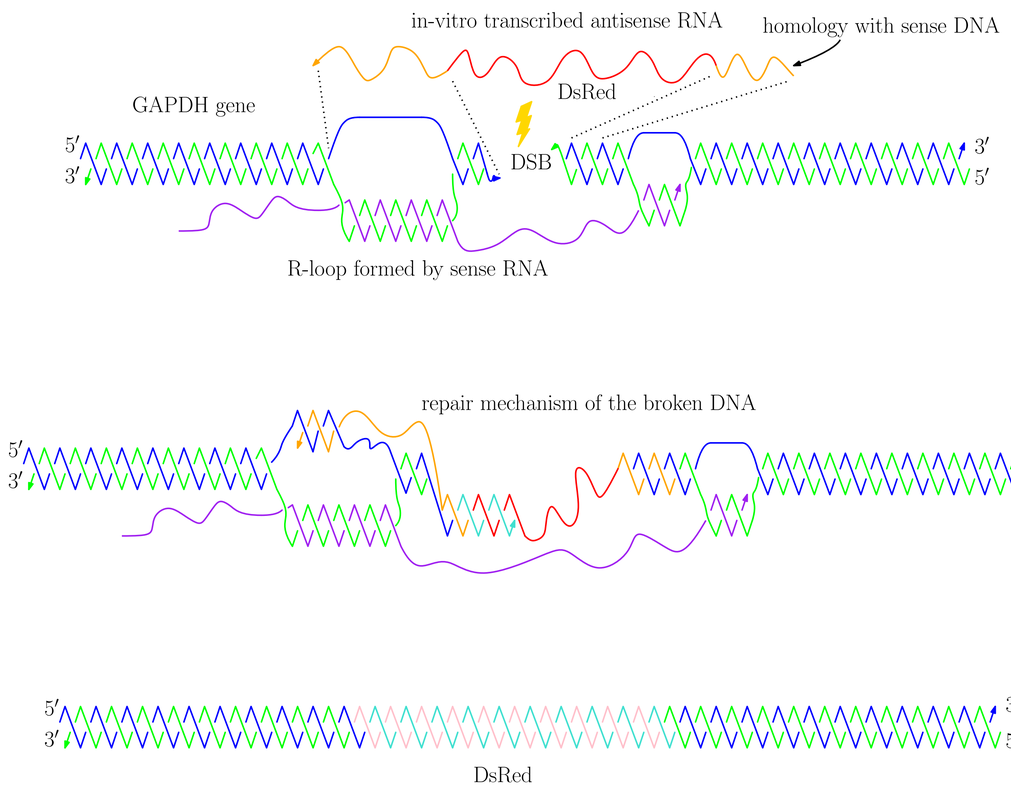

Figure 3: Schematic representation of the system to detect DSB repair in the presence of an R-loop.

Results

- We employed the software R-looper [3] to determine where R-loops may appear in the GAPDH gene. R-looper implements a statistical mechanical equilibrium model that takes into account DNA topology and base sequence to analyze R-loop formation.

  We used the GAPDH sense DNA with/without introns as input, together with different combinations of parameters to help with the experimental design:
  - N specifies the length of the DNA sequence that is not in its relaxed state;
  - *unconstrained* better represents linear DNA.

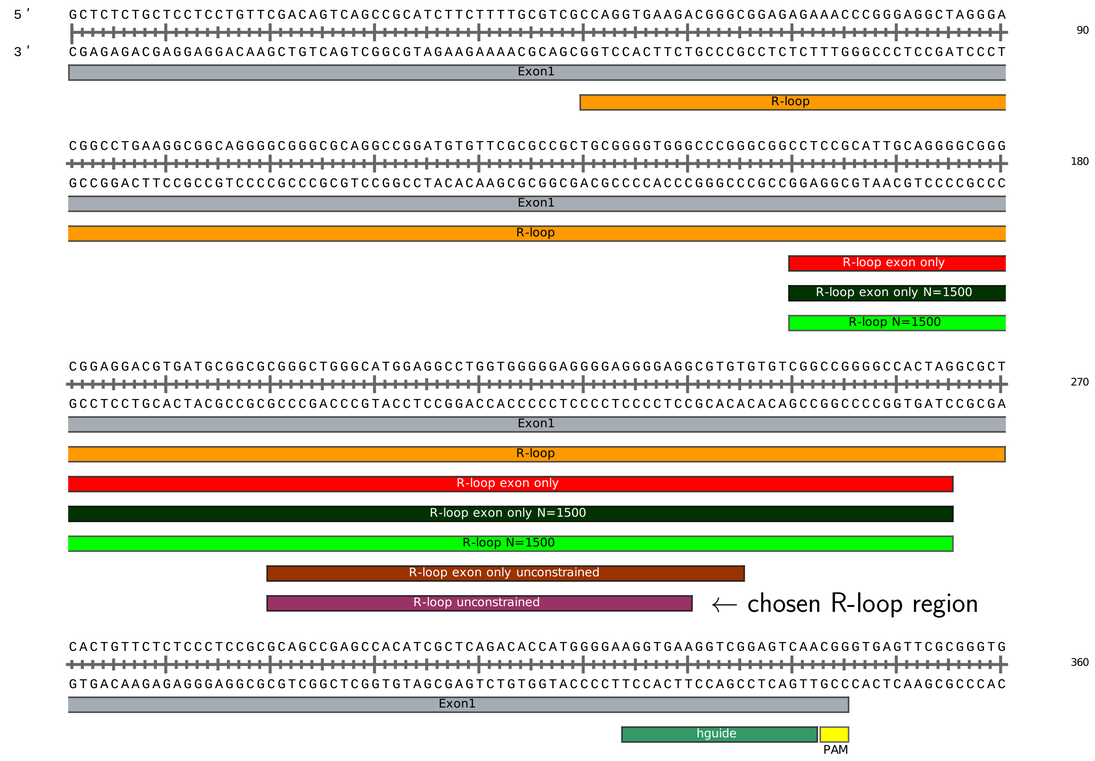

Figure 4: Snapshot of R-loop locations in GAPDH gene computed with R-looper.

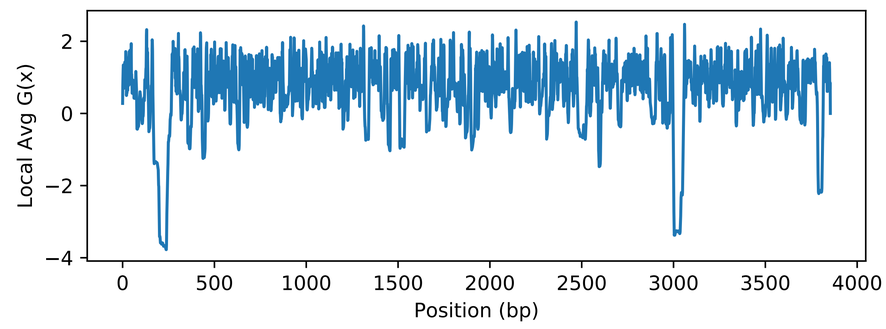

Figure 5: Energy value at each base pair computed using the "unconstrained" parameter.

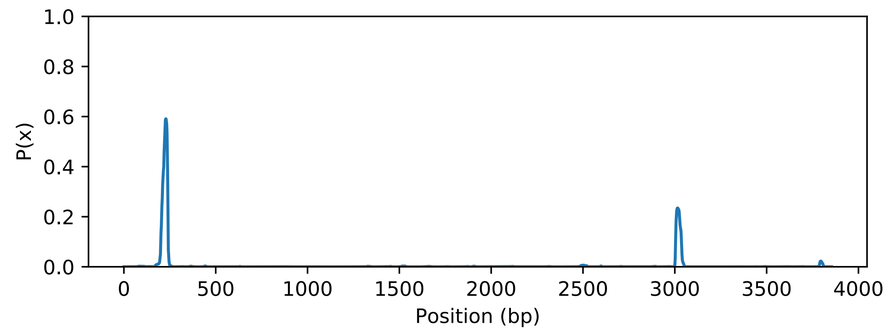

Figure 6: Probability of each base pair being found in an R-loop. The corresponding energy landscape is illustrated in Figure 5. The first peak represents the "purple R-loop" in Figure 4.

- In the experimental design the DSB is induced 3 base pairs (bp) upstream from the PAM sequence.
- The homology sequences of the in-vitro transcribed antisense RNA are 150 bp long.

With these settings, we hope to detect cells expressing red flurescence, showing that the DsRed has been properly incorporated into the GAPDH gene.

References

[1] H. Keskin, Y. Shen, F. Huang, M. Patel, T. Yang, K. Ashley, A.V. Mazin, F. Storici, *Transcript-RNA-templated DNA recombination and repair*. Nature **515**(7527), 436–439 (2014)

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[3] R. Stolz, S. Sulthana, S.R. Hartono, M. Malig, C.J. Benham, F. Chedin, *Interplay between DNA sequence and negative superhelicity drives R-loop structures*. Proceedings of the National Academy of Sciences **116**(13), 6260–6269 (2019)