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RNA–DNA hybrids: double-edged swords

RNA–DNA hybrids are associated with DNA damage and genome instability and are thus typically thought of as harmful. A paper in *Cell* now reports that RNA–DNA hybrids have a functional role in the homologous recombination (HR)-mediated repair of double strand DNA breaks (DSBs) and serve to protect genome integrity in yeast.

Ohle *et al.* sought to better understand the function of RNase H enzymes, which are known to degrade RNA–DNA hybrids. To this end, they generated RNase H deletion mutants of *Schizosaccharomyces pombe* and analysed their responses to DNA damage. Strains lacking RNase H were highly sensitive to drug-induced DSBs and contained increased levels of RNA–DNA hybrids. To enable further investigation, the authors engineered a system that allowed them to induce a chromosomal DSB at a specific cleavage site. Following DSB induction, cells lacking RNase H were seriously impaired in growth recovery and were unable to carry out DSB repairs.

The requirement for RNase H in HR-mediated DSB repair suggested an inhibitory role for RNA–DNA hybrids in the repair process. To explore this hypothesis, the researchers targeted RNA–DNA hybrids by overexpressing RNase H. This overexpression and presumed destabilization of RNA–DNA hybrids substantially delayed DSB repair and reduced the recovery rate of cells following DSB induction, similar to RNase H deletion, suggesting that RNA–DNA hybrids are a functional intermediate in the HR-mediated DSB repair pathway.

Seeking direct evidence for RNA–DNA hybrid involvement, the authors searched for hybrids in the vicinity of the cleavage site. Following the induction of DSBs, they detected a strong enrichment of RNA–DNA hybrids around the DSB; the enrichment was enhanced in cells lacking RNase H and was abolished by the overexpression of RNase H. Analysis of the position of RNA–DNA hybrids suggested that they result from transcription directed outwards from the DSB in both directions and subsequent transcript–template hybridization.

HR-mediated DSB repair involves resection of the 5′ strand on either side of the DSB, which leaves long single-stranded DNA (ssDNA) 3′ overhangs. These ssDNA stretches are bound and protected by replication protein A (RPA) before completion of the repair process, which occurs through invasion of homologous DNA on the sister chromatid. The authors reasoned that the ssDNA overhangs could serve as a template for the transcription generating RNA–DNA hybrids around DSBs.

Consistent with this model, induction of DSBs strongly increased RNA polymerase II (Pol II) levels around the cleavage site, indicative of active transcription at DSBs. In addition, the recruitment of RPA was strongly impaired in cells lacking RNase H and increased in cells overexpressing RNase H. Moreover, overexpression of RNase H greatly extended the length of RPA-enriched DNA, suggestive of extended 5′ strand resection as a result of RNA–DNA hybrid degradation. Together, these findings suggest that the presence of ssDNA at DSBs induces transcription, generating transient RNA–DNA hybrid intermediates that inhibit the speed and length of 5′ strand resection and RPA recruitment around DSBs.

Finally, analysis of small colonies that arose after DNA damage in strains overexpressing RNase H revealed a loss of repeat regions around the cleavage sites. This finding and further experiments suggested that RNA–DNA hybrids protect against unwanted recombination between repeat regions on sister chromatids during DSB repair. Thus, contrary to being merely harmful by-products of transcription, RNA–DNA hybrids appear to have important physiological roles in the regulation of DNA repair and the maintenance of genome integrity.

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ORIGINAL ARTICLE Ohle, C. *et al.* Transient RNA–DNA hybrids are required for efficient double-strand break repair. *Cell* **167**, 1001–1013 (2016)