DNA REPAIR

Transcriptionally tailored break repair

> …the integrity of actively transcribed loci is maintained by a dedicated DSB repair mechanism that involves the formation of R-loops and the recruitment of RAD52

The choice of repair of DNA double-strand breaks (DSBs) between the accurate homologous recombination repair (HRR) pathway and the error-prone non-homologous end joining (NHEJ) pathway is largely determined by the accessibility of nucleases that perform extensive DSB end resection and promote HRR. These nucleases and other HRR factors accumulate at DSBs in transcriptionally active regions, but the mechanism of HRR pathway choice at these sites remains unclear. Yasuhara and colleagues now show that accurate DSB repair at active genes is initiated by the recruitment of HRR factors to transcriptional DNA–RNA hybrids (R-loops).

To study the role of transcription in the repair of two-ended DSBs, the authors treated human epithelial cells with transcription inhibitors (TRis), including a transcription initiation inhibitor and the transcription elongation inhibitor DRB, before treating the cells with ionizing radiation, which induces DSBs. TRi treatment reduced the number of repair foci formed by the HRR factors RPA and RAD51 and the formation of sister-chromatid exchanges (SCEs), which is HRR-dependent.

Credit: Getty/NNehring

Furthermore, RPA recruitment to chromatin at a specific, experimentally induced DSB within a gene was transcription-dependent. Thus, active transcription is required to initiate HRR in a fraction of DSBs. The authors designated this transcription-associated HRR (TA-HRR) and estimated that 5–10% of the DSBs were repaired by this pathway.

The HRR factor RAD52 was previously implicated with RNA in DSB repair. Deletion of *RAD52* in human cells (Δ*RAD52*) reduced the formation of RPA and RAD51 foci and SCEs following irradiation, to the same extent as TRi treatment in wild-type cells. Importantly, RAD51 recruitment to DSBs at transcriptionally active but not at transcriptionally inactive loci was dependent on RAD52, indicating that RAD52 is crucial for TA-HRR.

Depletion of 53BP1 or RIF1, which promote NHEJ, restored RPA foci number in Δ*RAD52* cells, suggesting that they compete with TA-HRR factors functioning downstream of RAD52 for access to DSBs. In support of this, *RAD52* deletion or DRB treatment increased the frequency of NHEJ-mediated chromosomal aberrations. Analysis of breast cancer samples from The Cancer Genome Atlas revealed a negative correlation between levels of RAD52 expression and of genomic aberrations in gene loci. These data suggest that TA-HRR maintains the integrity of active genes.

The involvement of other HRR factors in TA-HRR was then investigated. Depletion of CtIP did not have an additive effect on the frequency of chromosomal aberrations in Δ*RAD52* cells, and depletion of BRCA1, which interacts with CtIP, did not have an additive effect on the reduction of RPA foci number in Δ*RAD52* cells. Indeed, BRCA1 was recruited to chromatin in a RAD52-dependent and CtIP-dependent manner; ATM-mediated BRCA1 and CtIP phosphorylation was also required for TA-HRR. Thus, BRCA1, CtIP and ATM function downstream of RAD52 in the TA-HRR pathway.

Published data indicated that RAD52 and R-loops are interconnected. Laser microbeam irradiation showed that both RAD52 and R-loops accumulated at irradiated chromatin within 60 seconds in a transcription-dependent manner. Moreover, RAD52 recruitment was R-loop-dependent and RPA foci number was reduced in cells over-expressing an R-loop resolving factor. R-loops consist of a DNA–RNA hybrid and a displaced single-stranded DNA. Pausing of RNA polymerases at DSBs could promote DNA strand displacement and the formation of DNA–RNA hybrids, which initiate TA-HRR by recruiting RAD52.

Finally, R-loop clearance was found to be delayed in Δ*RAD52* cells, and the endonuclease activity of the repair factor XPG was required for RAD52-dependent R-loop processing and TA-HRR. Conversely, XPG was dispensable in Δ*53BP1* cells, indicating that the resection activity of XPG counteracts 53BP1 function and NHEJ.

In summary, the integrity of actively transcribed loci is maintained by a dedicated DSB repair mechanism that involves the formation of R-loops and the recruitment of RAD52 and downstream HRR factors, which antagonize NHEJ and initiate the extensive end resection that is required for RPA and RAD51 recruitment. Owing to their location in active genes, misrepair of these DSBs due to low RAD52 expression levels favours NHEJ and could support oncogenesis.

Eytan Zlotorynski

ORIGINAL ARTICLE Yasuhara, T. et al. Human Rad52 promotes XPG-mediated R-loop processing to initiate transcription-associated homologous recombination repair. *Cell* https://doi.org/10.1016/j.cell.2018.08.056 (2018)