GENOMICS

High-resolution mapping of R loops

Two novel techniques refine genome-wide mapping of R loops.

The R loop is a nucleic acid *ménage à trois* in which RNA inserts between the two DNA strands, hybridizing with one and forcing the other to bulge out. Identifying the genomic location of R loops is important for understanding their function, but, current mapping methods offer limited resolution.

Now, Samie Jaffrey's group at Cornell University and Xiang-Dong Fu's laboratory at the University of California, San Diego have generated the two most precise R-loop-mapping techniques to date.

The Jaffrey group modified the DNA–RNA immunoprecipitation sequencing (DRIP-seq) technique, based on antibody-mediated capture of RNA–DNA hybrids from fragmented genomic DNA, followed by deep sequencing (Dumelie and Jaffrey, 2017). The resolution of DRIP-seq is limited by the size of immunoprecipitated DNA fragments. Thus, the researchers introduced a step preceding DRIP-seq that relies on cytosine-to-uracil conversion in genomic regions with single-stranded DNA. One DNA strand within the R loop is single stranded; thus, the R loop can be precisely mapped because of the exclusive presence of uracils. The results confirmed previous findings that most R loops are associated with promoters and suggested that the boundaries of R loops are defined by transcription start sites (TSSs) at the 5′ end and the first exon–intron junction at the 3′ end. In intronless genes, which are strongly associated with R loops, the TSS marks the 5′-R-loop border, whereas the 3′ boundaries are variable.

The R-ChIP approach developed by the Fu laboratory relies on the expression of a V5-tagged catalytically dead RNase H, which binds to but does not degrade the RNA in RNA–DNA hybrids (Chen *et al.*, 2017). The RNA–DNA hybrids are captured by the anti-V5 antibody, followed by chromatin immunoprecipitation. In agreement with previous findings, the R-ChIP experiments showed that most R loops map to active gene promoters and detected a greater association with open chromatin. The analyses also suggested that R-loop formation requires a nearby free RNA end and correlates with RNA polymerase II pausing at TSSs.

These new tools for R-loop mapping may provide exciting new insights into the (patho)physiological role of R loops.

Vesna Todorovic

RESEARCH PAPERS

Chen, L. *et al.* R-ChIP using inactive RNase H reveals dynamic coupling of R-loops with transcriptional pausing at gene promoters. *Mol. Cell* **68**, 745–747.e5 (2017).

Dumelie, J. G. & Jaffrey, S. R. Defining the location of promoter-associated R-loops at near-nucleotide resolution using bisDRIP-seq. *eLife* **6**, e28306 (2017).