The *Trypanosoma brucei* RNA/DNA hybrid interactomes reveals a role for RAD51 in R-loop homeostasis and repair of *VSG*-localised DNA breaks during antigenic variation

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The process of cell division requires the coordination of two often conflicting events that access the genome simultaneously: DNA replication and transcription. R-loops, which are three-stranded nucleic acid structures comprised of an RNA/DNA hybrid in the context of a displaced single-stranded DNA, usually arise when an elongating transcript reinvades the template DNA, but can also occur in *trans*. They can serve as obstacles during replication and can be sources of DNA damage. Because of this, R-loops are involved in many crucial processes in all organisms and are therefore under tight regulatory control. Mapping R-loops in the unicellular protozoan parasite *Trypanosoma brucei* revealed widespread enrichment, including in subtelomeric Variant Surface Glycoprotein (VSG) expression sites, linking them to DNA damage and antigenic variation (Briggs et al., 2018a; Briggs et al., 2018b). However, the mechanisms that control many aspects of R-loop biology in the *T. brucei* genome remain unclear. Using RNA/DNA hybrid immunoprecipitation coupled with mass spectrometry, 616 putative R-loop–interacting proteins were identified, including interactors with activities linked to RNA processing and DNA replication, repair, and recombination. To search for R-loop interactors with roles in antigenic variation, interactomes from bloodstream form and procyclic form parasites were compared. Of these proteins, four putative interactors were further investigated: two recombinases with known roles in VSG switching, RAD51 and RAD51-3, and two other proteins with unreported functions, a putative SNF2 chromatin remodeler and an ATP-dependent DEAD/H RNA helicase (DH). Loss of all proteins each led to nuclear genome damage and alterations in VSG expression dynamics. RNA/DNA hybrid immunofluorescence analysis revealed that loss of only RAD51 resulted in a global decrease in R-loop abundance, while depletion of SNF2, DH, and RAD51-3 led to a global increase. Genome-wide mapping of R-loop distribution in RAD51 mutants using DRIP-seq indicated depletions in R-loops at genomic sites including VSG-associated 70-bp repeats. Moreover, using Breaks Labelling *In Situ* and Sequencing (BLISS) we found pronounced levels of DNA breaks that localise to the 3’ end of the expressed VSG and become more abundant in RAD51 mutants. Our data reveal multiple unexplored activities that may influence R-loop function in the *T. brucei* genome and provide a mechanistic link between R-loops and the parasite’s ability to evade host immunity through VSG switching.

Briggs, E., Crouch, K., Lemgruber, L., Lapsley, C., & McCulloch, R. (2018a). Ribonuclease H1-targeted R-loops in surface antigen gene expression sites can direct trypanosome immune evasion. *PLoS Genetics, 14*(12). doi:10.1371/journal.pgen.1007729

Briggs, E., Hamilton, G., Crouch, K., Lapsley, C., & McCulloch, R. (2018b). Genome-wide mapping reveals conserved and diverged R-loop activities in the unusual genetic landscape of the African trypanosome genome. *Nucleic Acids Research, 46*(22), 11789-11805. doi:10.1093/nar/gky928