

Linking RPA and R-loops in *Trypanosoma cruzi*

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Trypanosoma cruzi, the etiological agent of Chagas disease, alternates between insect vectors and mammalian hosts, encountering diverse microenvironments and immune pressures. This lifestyle requires a balance between generating genetic diversity for adaptation and maintaining core genome stability. Although DNA replication is generally accurate, it can contribute to genome variability through replication-transcription conflicts, previously associated by our group with mutation hotspots in *T. cruzi*.

These conflicts are linked to DNA:RNA hybrids (R-loops), formed when nascent RNA hybridizes with the DNA template strand, displacing the non-template strand. Under physiological conditions, R-loops participate in transcription regulation, chromatin organization, and replication initiation, but their accumulation can stall replication forks and promote DNA damage.

Replication Protein A (RPA) is the main single-stranded DNA-binding complex involved in DNA replication and repair. In model systems, RPA associates with R-loops, preventing secondary structure formation and recruiting resolution factors.

While characterizing TcRPA-1 interactors by immunoprecipitation followed by mass spectrometry, we observed enrichment of ribosomal proteins, possibly reflecting interactions in rRNA-rich regions such as the nucleolus, a known hotspot for R-loop formation, as well as ALBA-family proteins, which modulate DNA:RNA hybrid stability. These findings led us to hypothesize that RPA may participate in R-loop regulation in *T. cruzi*.

We tested this hypothesis using immunofluorescence, revealing nuclear co-localization of three RPA subunits with R-loops, particularly after camptothecin treatment, a topoisomerase I inhibitor known to stabilize R-loops. Altogether, our results suggest that the RPA complex is recruited to R-loop-enriched regions in *T. cruzi*, supporting a potential role for RPA in R-loop-associated processes.