**Composition of the DNA replication fork in *Trypanosoma brucei***

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The eukaryotic replication fork is well-characterised in some model eukaryotes, but remains poorly understood in *Trypanosoma brucei*, where DNA replication faces several challenges. For instance, it is unclear how DNA replication proceeds in the context of multigenic transcription in the genome core relative to the transcriptionally silent subtelomeres or in hundreds of sub-megabase chromosomes. Investigation of the replisome and its assembly is needed to understand how DNA replication fidelity is maintained in the face of replication obstacles, both epigenetic and genomic-landscape related.

To define the replication fork proteome in *T. brucei*, TurboID proximity labelling was employed in unsynchronised bloodstream form cells under excess biotin conditions, utilising bait replisome components occupying distinct functional niches: PCNA, a sliding clamp of the replication fork, and the CMG (CDC45-$MCM_{2-7}$-GINS) replicative helicase subunits CDC45 and MCM2 - the latter of which also interacts with the Origin Recognition Complex (ORC) as the pre-replication complex. Turbo-tagged proteins displayed strong nuclear biotinylation patterns consistent with their expected cell-cycle localisation dynamics. Mass spectrometry identified robust enrichment of canonical replisome components, including $MCM_{2-7}$ subunits, DNA polymerases α, δ, and ε, and DNA ligase, supporting the recovery of genuine replication fork interactors by this study. Proteins associated with transcription, chromatin remodelling and DNA repair also showed strong presence in these data, consistent with a complex replication fork environment. We are now exploring the TurboID datasets to uncover so far uncharacterised *T. brucei* replication factors, including an MCM10 homologue. One of the most robustly recovered factors was a hypothetical protein that distant homology searches suggest to be a highly diverged Cdt1 candidate, providing the first evidence of how MCM is recruited to the diverged ORC at origins in *T. brucei*. Together, these data define an interaction network at the *T. brucei* replication fork, further validate core replisome machinery, and begin to identify parasite-specific variant DNA replication factors for future investigation in this divergent eukaryote.