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Direct Interaction Between ORC and Transcription Factors Directs DNA Replication Initiation in *Trypanosoma brucei*

Abstract text:

All organisms, including the causative agent of sleeping sickness *Trypanosoma brucei*, must precisely duplicate their genome before cell division to ensure faithful inheritance. DNA replication is a tightly regulated, multi-step process in eukaryotes; however, several core components of the replication machinery have diverged significantly in these early-branching parasites. Notably, the *T. brucei* origin recognition complex (ORC) differs from canonical eukaryotic ORCs by lacking Orc6, and containing an S-phase–enriched subunit, Orc1B. In addition, genome-wide studies have shown that the boundaries of multigene transcription units, known as strand switch regions (SSRs), are the locations of DNA replication initiation in the genome core. The molecular basis for this spatial overlap between transcription and replication remains unknown. To investigate whether transcription-associated factors contribute to ORC positioning and origin activation at SSRs, we systematically mapped the ORC proximal interactome using proximity-dependent biotinylation (TurboID) combined with quantitative mass spectrometry using three putative ORC subunits: Orc1B, Orc2, and Orc4. . Across all datasets, we identified 39 shared proximal proteins, 12 of which are transcription-associated factors. We validated physical associations between ORC subunits and four transcription-related proteins: TATA-binding protein (TBP), Bromodomain factor 2 (BDF2), YEA2 (the ortholog of the acetylated H2A.Z-binding proteins Yaf9/Gas41), and the SPARC complex component PWWP1. Integration of these interaction data with previously generated replication timing profiles and ChIP-seq analyses revealed distinct accumulation patterns at SSRs: TBP—the top interactor in the Orc1B proximity dataset—is preferentially enriched at early-replicating SSRs, whereas BDF2, YEA2, and PWWP1 display broader distribution across both early- and late-replicating SSRs. These findings strongly suggest that specific ORC–transcription factor interactions determine origin specification and activation timing. Collectively, our study provides the first evidence in eukaryotes of direct physical associations between ORC components and transcription factors, uncovering a mechanistic route by which origins are designated

for DNA replication initiation in *T. brucei*. Furthermore, these findings reveal how DNA replication programs are shaped in a divergent eukaryote.

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