HOW DO COMMON AND DIVERGED FEATURES OF THE REPLICATIVE STRESS RESPONSE SHAPE THE BIOLOGY OF TRITRYP PARASITES?

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ABOUT THE PROJECT

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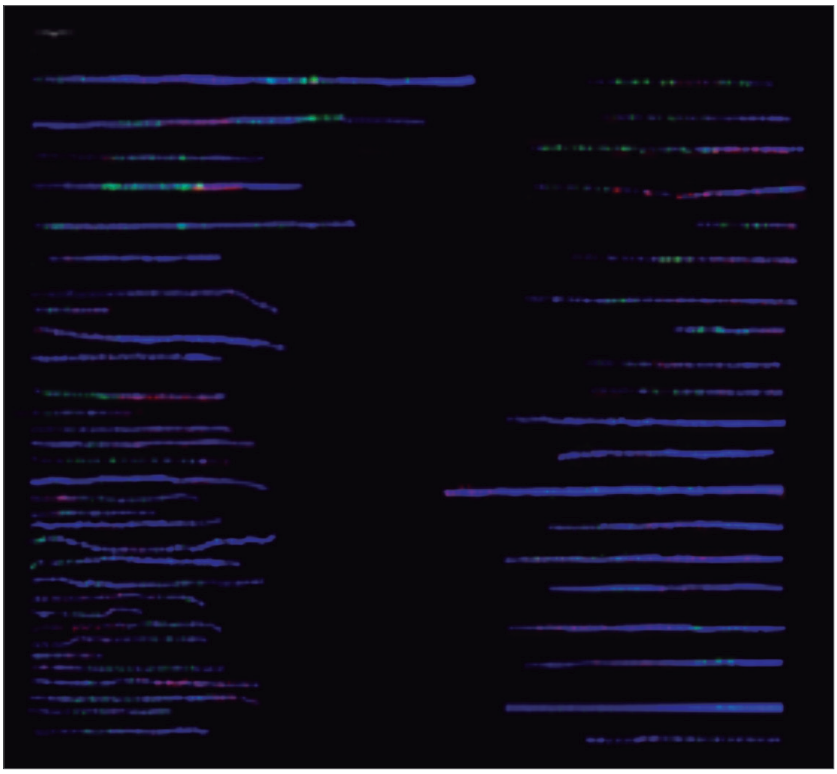
Kinetoplastids are eukaryotic microbes marked by diverged features of core eukaryotic biology. Nuclear genome sequencing has revealed that virtually all genes are grouped in directional gene clusters. In any organism, collisions between the DNA replisome and RNA Pol at genes transcribed during S phase are associated with genomic instability. The extent of the kinetoplastid genome that is transcribed, and the distances that RNA Pol II must traverse in a single direction suggest that such collisions must be pronounced in these genomes. In fact, replication origin mapping in *Trypanosoma brucei* and in *Leishmania* has shown that collisions between transcription and replication forks are not avoided. How the kinetoplastids *Trypanosoma cruzi, Trypanosoma brucei* and *Leishmania* (the 'tritryps') tackle such replication stress, and the implications of the resolution of such collisions for genome and parasite biology is the subject of this proposal.



Replication analysis after thymidine analog labeling. *Parasites that are replicating during the S phase of cell cycle incorporate the analogs IdU and CldU into their DNA during consecutive pulses. The incorporated analogs are immunodetected with specific antibodies and appear as red (IdU) and green (CldU) signals. The direction of fork replication follows the temporal order of the pulses, which is red followed by green. (Araujo et al., 2018, J Euk Microbiol, in press)*

SUMMARY OF RESULTS

Genomic plasticity and genetic variability in trypanosomatids are fundamental for the success of the infection process. Our data begin to reveal the molecular mechanisms involved in these processes. We found in *Trypanosoma cruzi* a preferential position of replication origins in regions where collisions between transcription and replication machinery are favored. In agreement with our hypothesis that these collisions are source of genetic variability, we found a positive correlation between origin location and single nucleotide polymorphism (SNP) accumulation. In *Leishmania*, we showed that HUS1 has a pivotal role in the way that this parasite handle with replicative stress, and its modulated expression is related with increment of SNPs when DNA replication is impaired. Finally, we could show that the transcription process is indeed a source of replicative stress in *Trypanosoma brucei* and the role of transcription as source of genetic variability is under investigation.

MAIN PUBLICATIONS

De Araujo CB, Calderano SG, Elias MC. 2018. The dynamics of replication in *Trypanosoma cruzi* parasites by single-molecule analysis. *J. Eukaryot. Microb*. In press.

Alves CL, Repolês BM, da Silva MS, Mendes IC, Marin PA, Aguiar PHN, Santos SDS, Franco GR, Macedo AM, Pena SDJ, Andrade LO, Guarneri AA, Tahara EB, Elias MC, Machado CR. 2018. The recombinase Rad51 plays a key role in events of genetic exchange in *Trypanosoma cruzi*. *Sci. Rep*. **8(1)**:13335.

Da Silva MS, Pavani RS, Damasceno JD, Marques CA, McCulloch R, Tosi LRO, Elias MC. 2017. Nuclear DNA replication in trypanosomatids: There are no easy methods for solving difficult problems. *Trends Parasitol*. **33 (11)**: 858-874.