Chromosomal DNA replication and segregation in Leishmania

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Résumé

In most biological models, reproduction as identical or similar organisms is based on the extreme accuracy of the mechanisms involved in the even transmission of the genetic material to the two daughter cells. This 'golden rule' does not seem to apply in Leishmania in which asymmetric chromosomal allotments during mitosis are responsible for a unique ploidy organization termed 'mosaic aneuploidy'. To get further insight into this unique feature, we developed an interest in chromosomal replication and segregation which are ill-known in Leishmania. We also studied the 'sister parasite' Trypanosoma brucei, which is diploid and where these key cellular processes are better elucidated. We followed two independent research approaches. First, to determine the physical parameters of the replication process, we analysed DNA replication dynamics in these parasites using DNA molecular combing; this allowed showing particularly large inter-origin distances and high speeds of DNA replication forks. Second, we studied the chromosomal dynamics during mitosis. Using fluorescent in situ hybridization combined with immunofluorescence in T. brucei procyclic forms, we determined the spatiotemporal dynamics of (i) the centromeres of chromosome II and III, and (ii) TbMlp2, the ortholog of a nucleoporin, during the course of the cell cycle. In interphase, the centromeres and TbMlp2 were located at the periphery of the nucleolus. TbMlp2 was then seen progressively migrating from the periphery of the nucleolus to the spindle poles. The position of the centromeres remained unchanged until TbMlp2 had completed migration to the spindle pole; then the centromeres themselves started migrating to the poles. In addition, RNAi knockdown of TbMlp2 lead to aneuploidy. Altogether, these data suggest that, unexpectedly, TbMlp2 may play a key role, as a molecular chaperone and/or transport protein, in the dynamics of chromosomal segregation. In total, both approaches again revealed original features in these divergent eukaryotes as compared to classical models.

Mots-Clés: Parasitologie, Aneuploïdie, FISH, Peignage moléculaire, Réplication, Ségrégation, Kinétochores, Eucaryote divergent

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