The mitochondrial genome and gRNA-ome of pleomorphic *Trypanosoma brucei*

Sinclair Cooper, Nick Savill, Torsten Ochsenreiter, Achim Schnaufer

We present the first near-complete mitochondrial genome (kDNA) and corresponding small RNA transcriptome of *T. brucei.* The kDNA (or kinetoplast) represents the most complex mtDNA in nature. It is composed of ~23-kb maxicircles (~50 homogenous copies), analogous to mitochondrial genomes of other eukaryotes, and thousands of highly heterogeneous ~1-kb minicircles, unique to kinetoplastida. Maxicircles encode subunits of respiratory chain complexes as well as a mitoribosome subunit and rRNAs. mRNAs for 12 maxicircle genes require post-transcriptional RNA editing to become functional, a process mediated by minicircle-encoded guide RNAs (gRNAs). We have now generated the first nearly complete (~99%) kDNA genome assembly of *T. brucei*, including predictions of gRNA genes and associated minicircle motifs, and determined the small RNA transcriptome before and after differentiation from bloodstream to procyclic form. We show that many minicircle genes encode transcripts that do not match sequences in the known editing space. These gRNA-like molecules bear many of the characteristics of known gRNAs, but also have distinct features, suggestive of a functionally distinct role. Although the life cycle stages of *T. brucei* in the mammal and in the tsetse fly depend on different subsets of mitochondrial mRNAs, we find that both life cycle stages express sufficient gRNAs to cover the entire known editing space.