Inhibiting the essential F_oF_1-ATPase activity in the infectious form of the human pathogen, *Trypanosoma brucei.*

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The flagellated protist, *Trypanosoma brucei*, is a lethal human pathogen that alternates between a mammalian host and its tsetse fly vector. Therefore, this extracellular parasite must adapt its bioenergetic processes while encountering diverse carbon sources as it progresses through its complex life cycle. In the insect or procyclic stage (PS), the predominant metabolites are proline and threonine, which are used to generate ATP by the typical oxidative phosphorylation (OXPHOS) pathway. In this life stage, the robust and highly branched singular mitochondrion is fully developed with abundant cristae, a complete electron transport chain (ETC) and active Krebs cycle enzymes. This is in stark contrast to the reduced cellular volume of the tubular mitochondrion present in the infectious or bloodstream stage (BS), which exploits the abundant glucose in the mammalian blood to synthesize ATP through the less efficient process of glycolysis. This metabolic switch from oxidative phosphorylation to glycolysis coincides with the loss of most mitochondrial cristae, the cytochrome-mediated respiratory chain and citric acid cycle enzymes.

Despite this diminished activity and simplified architecture, the BS mitochondrion still requires the expression of mitochondrial (mt) DNA and it harbors vital processes that are largely performed by nuclear encoded subunits that are imported into the mitochondrion. Thus, a mitochondrial membrane potential ($\Delta\Psi_m$) must be generated across the inner mt membrane. Since the proton-pumping components of the ETC are absent, the $\Delta\Psi_m$ is maintained by the hydrolytic activity of the F_oF_1-ATP synthase. This reversible function of complex V is indispensable for BS trypanosomes and can be inhibited by natural and synthetic inhibitors. Fortuitously, almost all eukaryotes encode the natural peptide inhibitor, IF1, whose function in mammalian cells has been described to bind F_oF_1-ATPase and obstruct the futile hydrolysis of ATP under hypoxic conditions. Therefore, we employed several bioinformatic tools to identify a diverged homolog of IF1 in the *T. brucei* genome, called TbIF1. Utilizing several *in vitro* assays, we have defined regions of TbIF1 that are crucial for the inhibition of the parasitic F_1-ATPase and described the TbIF1 inhibitory capacity, oligomerization, and pH sensitivity. Furthermore, we have demonstrated that TbIF1 expression is developmentally regulated, as it is expressed in PS trypanosomes but undetectable in BS cells. The significance of this regulation is articulated when ectopic TbIF1 over-expression is induced in BS *T. brucei*, triggering a collapse of the $\Delta\Psi_m$ that leads to the death of the infectious stage of the parasite. Since the human activity of this complex is to synthesize ATP under aerobic conditions, it is feasible to selectively disrupt just the parasitic F_oF_1-ATPase activity and possibly treat Human African Trypanomiasis.

Since the *T. brucei* F_oF_1-ATPase apears to be a good drug target, we delved deeper into the composition and structure of this rotary motor, which is highy conserved throughout the model opisthokonts. Intriguingly, 14 of the 22 identifed subunits of the *T. brucei* enzymatic complex have no orthologues outside of Euglenozoa. In fact, one of these subunits has been verified as a novel and essential component of the F_1 subcomplex, since its depletion leads to a growth defect as the catalytic F_1 domain becomes destabilized and inactive. Another unique attribute of this parasitic molecular machine is the *in vivo* proteolytic cleavage of subunit alpha into two fragments, both of which stay tightly associated with the complex. We are currently exploring the biological relevance of this specific cleavage because there is one subspecies of *T. brucei* that has managed to discard both its mt DNA and the N-terminal alpha cleavage peptide that is normally responsible for binding OSCP, a peripheral stalk subunit that acts to cap the catalytic F_1 moiety in all eukaryotes studied so far. These extraordinary features of the *T. brucei* F_1-ATPase break the long-standing conception that the architecture of the F1-ATPase complex is strictly conserved in eukaryotes.