Use of RNAi in the validation of potential Kinetoplastidae drug targets

Mantovani, M.[1]; Sturm, N.[2]; Campbell, D.A.[2] & Thiemann, O.H.[1]

1. Laboratory of Protein Crystallography and Structural Biology, IFSC-USP, Av. Trabalhador Sãocarlense 400, PO Box: 369, São Carlos/SP, Brazil.
2. Microbiology, Immunology & Molecular Genetics Department, UCLA, 1602 Molecular Science Building Box 951489, Los Angeles/CA, USA.

RNA interference (RNAi) is the mechanism through which gene-specific double-stranded RNA (dsRNA) triggers degradation of homologous transcripts and it has been used as an invaluable tool to down regulate gene expression in a variety of organisms. In protozoan parasites, the RNAi mechanism is present in *Trypanosoma brucei*, whereas other members of the same family, such as *T. cruzi* and *Leishmania major*, appear to be RNAi negative. Many species of *Leishmania* are responsible for serious visceral or skin diseases that exhibit high incidence in tropical and subtropical regions. The drugs currently employed in the treatment and in the control of parasitic diseases are potentially mutagenic and/or carcinogenic and often require prolonged treatment with multiple drug administration. These undesirable responses demonstrate the need for the development of alternative antiparasitic drugs. To undertake an effective program of drug design, the detailed knowledge of fundamental biochemical differences between the parasite and its vertebrate host as well as the validation of the potential therapeutic targets are essential. In this context, one of the most striking metabolic discrepancies, between some Trypanosomatidae and their human hosts, is the purine nucleotides biosynthesis pathway. Adenylosuccinate lyase (ADSL) is a bifunctional enzyme that catalyses two non-sequential steps in this cycle. ADSL from *L. major* has already been molecularly and biochemically characterized in our project. Hence, this work aimed to validate this enzyme as a potential target using the RNAi technique. We have cloned a fragment of the *adsl* gene from *T. brucei* into the tetracycline-regulated vector pZJM, which is appropriated to express dsRNA in this organism. The recombinant plasmid was then used to transfect procyclic *T. brucei* strain 29-13, which harbors integrated genes for T7 RNA polymerase and the tetracycline repressor. Transfected cells were grown in medium supplemented with G418 and hygromycin and stable transfectant cells were obtained by adding the selective antibody phleomycin. For induction of RNAi, cells were cultured in the presence of tetracycline and growth curves for non-induced (control) and induced cultures were obtained. The ADSL is shown to be essential for cell viability. With the purpose to confirm these results, we are analyzing the mRNA and protein extracts from the control and RNAi induced cells.