MFS Transporters in African trypanosomes: looking for a function.

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The trypanosomiases cover several parasitic diseases that are found in Africa, South Asia and Latin America. In *Trypanosoma brucei*, drug sensitivity and resistance depends on transporters mediating the uptake and/or efflux of chemotherapeutic agents.

For example, in *T. brucei* the TbAT1/P2 aminopurine transporter is involved in the uptake of diamidine and arsenical drugs including pentamidine, diminazene aceturate and melarsoprol whilst a loss of TbAT1/P2 and HAPT1 gives a high pentamidine-melarsoprol cross-resistance phenotype in *T. b. brucei*. The related parasite *T. congolense*, has a major amplification of the ENT family (up to 19 members), but phylogenetically most of these cluster as nucleobase transporters rather than nucleoside transporters (P1-cluster) or nucleoside/nucleobase transporters (P2 cluster). As such, *T. congolense* does not have a counterpart of TbAT1 and as a result it is much less sensitive to diminazene, although this is the main drug for the treatment of *T. congolense* infection.

Another important class of transporters in chemotherapy mediates efflux of metabolites. Among the most promising drug targets in trypanosomatids are cAMP Phosphodiesterases (PDEs); inhibitors of these enzymes prevent cAMP degradation, leading to toxic levels of cAMP in the cell. However, the effectiveness is limited by a mechanism exporting excess cAMP from the cell. Thus, the efflux transporter for cAMP is important in the pharmacology of PDE inhibitors, and in understanding cAMP signaling in the trypanosome. The aim of the current project is to find *T. congolense* transporter genes (and their orthologues in *T. brucei*) that drive drug resistance and or cAMP efflux. For this we commenced the first study of Major Facilitator Superfamily (MSF) transporters in trypanosomes.

Three *T. congolense* MFS transporter proteins (MF 5.2, 7.1 and 18.8) caused significant increases in resistance to Pentamidine in *T. brucei* clone ISMR1, a clone adapted to high levels of resistance to isometamidium but sensitive to pentamidine suggesting a possible efflux mechanism. Both alleles of the syntenic *T. brucei* genes were knocked out using homologous recombination and functionally characterised. The phenotypes of each of the gene deletion mutants will be presented. The cellular localisation of these MFS transporters is being assessed using fluorescence microscopy.

This study constitutes the first attempt to determine functions for some of the MFS transporters expressed by trypanosomes.