Heterologous expression of *Trichomonas vaginalis* Equilibrative Nucleoside Transporter Family Members in *Trypanosoma brucei*

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Trichomoniasis is the most common of the non-viral sexually transmitted diseases (STD); it is caused by the amitochondriate protozoan *Trichomonas vaginalis*. Increased resistance of *T. vaginalis* to metronidazole, the drug of choice for the treatment of the disease, necessitates the development of newer chemical entities with different chemotypes. The nucleoside/nucleobase salvage system of the parasite is an attractive target, because the parasite cannot synthesise either purines or pyrimidines *de novo* and has to salvage the nutrients from the host through transporters, whereas their human hosts have both purine salvage and synthesis pathways for purines and pyrimidines. Therefore, depriving the parasites of these essential requirements, through controlled blockage of the salvage transporters or pathways, is certain to cause parasite death, but should not affect the human host. Nucleoside salvage in the parasite was therefore systematically investigated using an array of radiolabelled nucleosides and nucleobases. The results show the existence in *T. vaginalis* of at least four transporters: with high and low affinity for purine and pyrimidine nucleosides, as well for as adenine, similar to that of transport activities previously characterised in other protozoans. In order to match the observed transport activities to specific genes, all 9 *T. vaginalis* Equilibrative Nucleoside Transporter (ENT) genes were cloned from cDNA, sequenced, and expressed in *Trypanosoma brucei*, selecting a strain from which one of the main nucleoside transporters, TbAT1, was already deleted. Each gene was resynthesized in the codon preference of *T. brucei* since *T. vaginalis* DNA. Two identical sets of transfectants have been constructed: one with synthetic but otherwise original open reading frames, and one with the ORFs coupled C-terminally to 6xHA tags to assess cellular localisation of the gene products. Immunofluorescence has demonstrated that the transporters are correctly routed to the cell surface, and qRT-PCR has shown substantial levels of expression.