

Novel nucleotidases involved in *Trypanosoma brucei* pyrimidine homeostasis.

Nucleotide metabolism has been an area of interest for the discovery of novel targets against many diseases since a balanced pool of deoxyribonucleotides is required for correct DNA replication and repair. Particularly relevant for cell survival is the maintenance of a balanced dUTP/dTTP ratio in the pyrimidine pool as several DNA polymerases cannot distinguish between the two nucleotides and incorporate indiscriminately one or the other depending on their availability. We have previously shown that thymidine kinase (*TbTK*) has a major role in the maintenance of the dUTP/dTTP ratio and the response to genotoxic agents in bloodstream forms of *Trypanosoma brucei*. We reported that *TbTK* was essential for parasite viability, both *in vitro* and *in vivo* thus demonstrating that phosphorylation of deoxyuridine and/or thymidine is important for the maintenance of the dTTP pool even in the absence of a source of extracellular pyrimidines. These observations indicated a role of the enzyme in *do novo* synthesis and pointed towards the existence of an intracellular deoxynucleoside pool available for phosphorylation. Here we have aimed at characterizing nucleotidases involved in the generation of intracellular nucleosides important for thymidylate *de novo* biosynthesis. We present data for HD domain containing nucleotidases with regard to their intracellular localization, role in cell viability, nucleotide pools and cell cycle progression and propose this class of enzymes as relevant players in nucleotide homeostasis in trypanosomes.