Cytidine deaminase in *Trypanosoma brucei*: a mitochondrial enzyme involved in *de novo* biosynthesis of pyrimidines.

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Trypanosoma brucei possesses the metabolic machinery for *de novo* synthesis of pyrimidine nucleotides but also has the capacity to obtain pyrimidines from the host via the salvage pathway. This parasite lacks the dCMP deaminase responsible for dUMP formation present in mammalian cells however it does contain a putative cytidine deaminase (TbCDA). We previously reported that *T. brucei* deoxyuridine triphosphate nucleotidohydrolase null mutants are thymidine (dThd) auxotrophs although 5-methyl-2’-deoxycytidine (5mdC) can also support growth presumably through deamination to yield dThd thus suggesting an important role of TbCDA in pyrimidine nucleoside homeostasis. We have characterized recombinant TbCDA and show that it catalyzes deamination of cytidine (Ctd) or deoxycytidine (dCtd) to uridine (Urd) and deoxyuridine (dUrd) respectively. TbCDA is also capable of deaminating several nucleoside analogues, such as 5mdC. In agreement with this observation, *T. brucei* bloodstream forms overexpressing TbCDA are hypersensitive to the analogue 5fdC. Cellular localization studies revealed that TbCDA is a mitochondrial enzyme in both procyclic and bloodstream forms of the parasite. RNAi-mediated depletion of the enzyme in *T. brucei* bloodstream forms resulted in defective growth in the absence of external pyrimidines. The defective growth phenotype is reversed by dThd supplementation further supporting the notion that TbCDA has an important role in providing dUrd for *de novo* dTMP biosynthesis.