

The curious origin and evolution of kinetoplastid glycosyltransferases

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The synthesis of glycoconjugates is a particularly common feature of protozoan parasites to facilitate interactions with, and provide protection from, their host environment. Many tenets of *N*- or GPI-anchor glycosylation shared amongst eukaryotes are highly conserved in kinetoplastids, but these parasites exhibit peculiarities in the process of glycosylation, including the evolution of a large GT67 glycosyltransferase (GT) family. This presentation will summarise our current knowledge into the process of glycosylation in these organisms, and discuss the evolutionary divergence of the GT67 family and their contribution to complex *N*-glycan and GPI-sidechain glycan branch synthesis. Phylogenetic analysis of the kinetoplastid specific GT67 gene family reveals their separation into two distinct clades for *Leishmania* and African trypanosomes, indicative of the different selective pressure exerted on their carbohydrate biosynthesis. Further, investigations of the GT67 family in *T. brucei* indicate the significant expansion and repurposing of this ancestral, eukaryotic β 3-glycosyltransferase into seven distinct TbGT gene lineages, presumably to facilitate complex glycan biosynthesis. Despite their evolution from a shared ancestor, the enzymes encoded by these seven GT67 gene lineages display comparatively low amino acid sequence identity. Experimental characterisation of their glycosyltransferase activity reveals they unexpectedly catalyse β 1-2, β 1-3 and β 1-6 glycosidic linkages, and utilise UDP-Galactose and UDP-*N*-acetylglucosamine as donor sugars. We will also highlight the discovery of an entirely novel and essential process of mitochondrial fucosylation in kinetoplastea and present recent investigations in *T. brucei* to assess the role of the GT11 family fucosyltransferase, TbFUT1. We will discuss the curious origin of TbFUT1, where recent phylogenetic analysis implicates the acquisition of the GT11 family gene from bacteria by horizontal transfer from a large, cytoplasmic DNA virus early in the evolution of the kinetoplastea. We propose the further acquisition of a bacterial GT25 family glycosyltransferase gene which, unlike GT67 family sequences, is highly conserved amongst kinetoplastids. Subsequent gene duplication has occurred in the kinetoplastids to encode two GT25 family enzymes, termed GTX and GTZ, with mitochondrial localisation but likely different glycosyltransferase activities. We aim to further investigate the function of mitochondrial glycosylation by identifying the substrate(s) of TbFT and characterising the activity of these bacterial-like, mitochondrial GTs.