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Roles of Protein GPI-Anchors in African Trypanosomes and Hosts

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Many eukaryotic proteins are anchored to the cell surface membrane by glycosylphosphatidylinositol (GPI). GPI is synthesized by stepwise additions of sugars and other components to phosphatidylinositol in the endoplasmic reticulum (ER) and transferred to proteins. A common backbone of GPI is conserved in yeast, protozoa and mammals, whereas differences exist in side-chains and the lipid part. Since GPI-anchored proteins are abundant in protozoan parasites and are essential for growth of some of them, such as bloodstream form of *Trypanosoma brucei*, the biosynthetic pathway of GPI-anchored proteins is considered to be a target of anti-protozoa drugs.

Precursor proteins destined to be GPI-anchored have the C-terminal signal sequence for attachment of the GPI anchor. When the precursor proteins are translocated into the lumen of the ER, the GPI transamidase recognizes the GPI attachment signal sequence and exchange it with GPI. The GPI transamidases of humans and *Saccharomyces cerevisiae* are similar complexes of five proteins, human components being GAA1, GPI8, PIG-S, PIG-T and PIG-U. GPI8 is most likely a catalytic subunit because it has homology to the cysteine proteases of the C13 family. PIG-T is critical for the formation of the enzyme complex. The roles of GAA1, PIG-S and PIG-U are yet to be clarified.

Trypanosoma brucei proliferates in the bloodstream of mammalian organisms and causes sleeping sickness. This parasite is transmitted by blood-sucking tsetse flies. Tsetse flies are distributed in the "tsetse belt" in central Africa where trypanosomiasis is a serious problem. Effective therapeutic and protective measures are highly desirable.

T. brucei has two distinct proliferative forms, a bloodstream form living in mammalian bloodstream and a procyclic form living in the midgut of tsetse fly. The surface of these forms of the parasite is covered by a large amount of GPI-anchored proteins: variant surface glycoproteins (VSG) in the bloodstream form and procyclins in the procyclic form. Bloodstream form *T. brucei* evades the mammalian immune response by exchanging the expressed isoform of VSG. Procyclic form is thought to protect itself from trypanocidal factors in the midgut by means of GPI-anchored procyclin coat.

Since the GPI attachment signal is not interchangeable between *T. brucei* and humans, we expected that their GPI transamidases may be different. We have isolated GPI transamidase of *T. brucei* and found that it is in fact different from the human enzyme: *T. brucei* GPI transamidase had three common components, GAA1, GPI8 and PIG-T, whereas it did not contain PIG-S and PIG-U but instead had two unique components. These unique components should be good targets of trypanosome-specific inhibitors of GPI transamidase. We have also found that GPI-anchors rather than the protein portion of procyclins are critical for survival of procyclic form in tsetse flies. Especially, addition of sialic acid to the side-chain of GPI by trans-sialidase is important. I will discuss recent data on these points.