Point of VIEW

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Designing Sleeping Sickness Control

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ABSTRACT Control of African trypanosomiasis caused by the protozoan parasite *Trypanosoma brucei* is an important issue in medicine, veterinary medicine, and agricultural economy. Because vaccine development is unlikely, development of safer and more effective chemotherapeutics is critical. The biosynthetic pathway of glycosylphosphatidylinositol (GPI), which acts as membrane anchors of coat proteins, variant surface glycoproteins, and transferrin receptors, is a validated target of drug development. An article in this issue reports the first chemically synthesized inhibitor of the third mannosyntransferase from the GPI pathway, stimulating further investigation toward practical and useful compounds.

African sleeping sickness in humans and the related disease Nagana in livestock cause important medical and agro-economical problems in >30 sub-Saharan African countries. Transmitted by blood-sucking tsetse flies, the African trypanosome (*Trypanosoma brucei*) causes at least 50,000−70,000 cases of sleeping sickness every year. These diseases are fatal if not treated properly (*1*). Ferguson and colleagues report on page 625 of this issue their chemical biology approach to inhibitors of an essential biosynthetic pathway of this parasite (*2*).

A vaccine against sleeping sickness is unlikely because of its antigenic variation (*3*). The surface of the bloodstream-form trypanosome that causes the disease is coated by 10 million molecules of a single protein, termed variant surface glycoprotein (VSG) (Figure 1, panel a). The bloodstream-form parasites injected into mammalian tissue *via* tsetse fly bites are protected from the complement of the mammalian immune system by the VSG coat. VSGs are immunodominant antigens that induce antibody response in the mammalian host. The bloodstream-form trypanosomes proliferating in the bloodstream are easily killed by anti-VSG antibody plus complement. *T. brucei* has hundreds of genes of VSG family members that have different antigenic properties. Almost all cells in the population express the same isoform of VSG at the same time and are killed as a result of the initial antibody response. However, some cells in the population switch the expressed VSG gene to another one, generating parasites resistant to the initially produced antibody, in turn resulting in recurrent proliferation of the surviving parasites. This process may be repeated until the parasites gain access to the central nervous system and cause fatal neurological problems.

Some antitrypanosome drugs are available; however, they have severe side effects and are not effective once parasites are in the central nervous system. Clearly, the development of safer and more effective chemotherapeutic agents is highly desirable to better treat African trypanosomiasis (*4*).

VSGs are glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) (*5*) (Figure 1, panel a). GPI is a complex glycolipid used as a membrane anchor of many cell-surface proteins in all eukaryotic cells. GPI-APs are particularly abundant in *T. brucei*. Not only VSGs but also transferrin receptors important for ion uptake are GPI-APs. GPI is synthesized in the endoplasmic reticulum from phosphatidylinositol by sequential reactions, including transfer of *N*-acetylglucosamine, de-*N*-acetylation of *N*-acetylglucosamine, and transfers of three mannoses, palmitic acid, and ethanolaminephosphate (Figure 1, panel b). GPI, which has an amino group of ethanolaminephosphate at the end, is then transferred all together as a post-translational modification to the carboxyl-terminus of proteins bearing a GPI attachment signal sequence. This occurs by means of transamidase-mediated exchange of the signal peptide and GPI (*6*).

When GPI biosynthesis is defective, proteins are not membrane-anchored but

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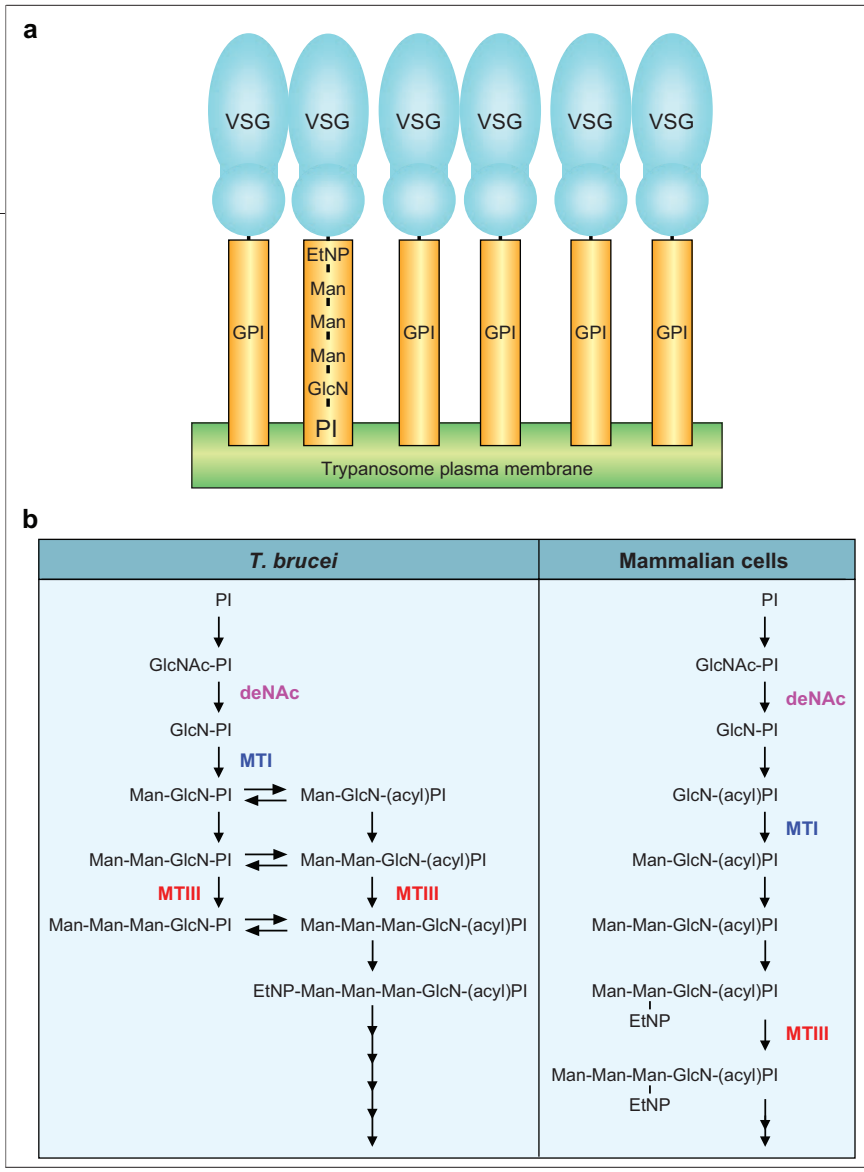

Figure 1. GPI-anchored variant surface proteins coat the plasma membrane of *T. brucei*. a) Schematic representation of the surface coat of *T. brucei* consisting of 10 million molecules of GPI-anchored VSG. PI, phosphatidylinositol; GlcN, glucosamine; Man, mannose; EtNP, ethanolaminephosphate. b) Comparison of *T. brucei* and mammalian GPI biosynthetic pathways.

rather are either secreted into extracellular compartment or degraded intracellularly. Therefore, if biosynthesis of GPI is inhibited, the VSG coat would not be formed properly and transferrin receptors would not be expressed on the surface membrane. It is known that GPI biosynthesis is essential for the growth of bloodstream-form *T. brucei* even in culture medium. Thus, gene knockout studies demonstrated that *TbGPI10* encoding α1-2 mannosyltransferase (MTIII), which mediates transfer of the third mannose, and *TbGPI12* encoding *N*-deacetylase (deNAc), which converts *N*-acetylglucosaminyl phosphatidylinositol to glucosaminyl phosphatidylinositol, are essential genes (*7, 8*) (Figure 1, panel b). It is likely that the VSG coat is important not only for protection from complement but also for physical integrity of the cell. A lack of transferrin receptors may also contribute to lethality. Therefore, GPI biosynthetic pathway is a genetically validated target of antitrypanosome drug development (*9*).

Ferguson and colleagues have been playing major roles in determining substrate specificities of GPI biosynthetic enzymes of *T. brucei* aiming to design and generate analogs of natural substrates inhibitory to GPI biosynthesis (*10–12*). GPI biosynthetic enzymes are all bound to the endoplasmic reticulum membrane and bear one or more transmembrane domains. Some of them are multisubunit complexes. Because these characteristics make enzyme purification for structural analysis difficult, structural data on their catalytic sites are not available. The general approach being taken by Ferguson and colleagues is to use lysates/membrane fractions of bloodstream form *T. brucei* and human HeLa cells as sources of GPI biosynthetic enzymes of the parasite and host and to test chemically synthesized substrates and their analogs for their abilities to act as acceptor substrates or inhibitors. Enzyme reactions are monitored by adding radiolabeled donor substrates or their precursors that can be converted to the donor substrates by membrane-associated enzymes and necessary components.

In previous years, Ferguson and colleagues studied enzymes involved in early reaction steps, namely, deNAc, which generates the second intermediate glucosaminyl phosphatidylinositol, and α1-4 mannosyltransferase (MTI), which transfers the first mannose (Figure 1, panel b). Substrate specificities of *T. brucei* and human deNAc are significantly different, and the authors designed two potent parasite-specific inhibitors, 2-deoxy-2-ureido-D-Glcβ1-6D-myo-inositol-1-HPO_4-*sn*-1,2-dipalmitoylglycerol and 2-deoxy-2-ureido-D-Glcα1-6D-(2-*O*-octyl)myo-inositol-1-HPO_4-*sn*-1,2-dipalmitoylglycerol. The IC_{50} of both compounds against *T. brucei* deNAc was ~8 nM, whereas they were not inhibitory to human deNAc up to 100 μM (*11*). *T. brucei* and human MTI also have different substrate specificities, reflecting different steps in which the first mannose is transferred. In the mammalian pathway, mannose transfer is preceded by palmitoylation of inositol, whereas in the *T. brucei* pathway, mannose is transferred before inositol-palmitoylation (Figure 1, panel b). Smith and colleagues found that glucosaminyl-(2-*O*-hexadecyl) phosphatidylinositol selectively inhibited trypanosomal MTI (*9*). More recently, Ferguson and colleagues showed that one cell-

permeable glucosaminyl phosphatidylinositol analog in fact killed bloodstream-form *T. brucei*, an indication that the GPI biosynthetic pathway is chemically validated as a drug target (*12*).

Ferguson and colleagues went further with their studies on enzymes in later steps in GPI biosynthesis and now report substrate specificity of *T. brucei* MTIII and the first chemically synthesized MTIII inhibitor (*2*). *T. brucei* MTIII did not recognize the diacylglycerol moiety of GPI and efficiently used a substrate analog (ManManGlcN-IPC_{18}) bearing a phosphate-linked C18 alkyl chain instead of diacylglycerol. Hydroxyl groups in the second mannose of ManManGlcN-IPC_{18} were then variously modified to deoxy, amino, or fluoro forms and tested for their abilities to act as MTIII substrate and to inhibit MTIII activity. Among them, the 2′-amino analog (one bearing amino group at 2′-position of second mannose) did not act as MTIII substrate as expected because MTIII transfers mannose to the 2′-position of second mannose. This analog inhibited MTIII with an IC_{50} of 1.7 μM.

Although the high-resolution structure is not known for *T. brucei* MTIII, this study by Ferguson and colleagues has successfully shown that a chemical biology approach is powerful and useful for identifying inhibitory substrate analogs, and it has demonstrated that *T. brucei* MTIII is an attractive target of chemotherapy against African sleeping sickness.

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