Subject Categories: [To be completed by editor]

Subject strapline: [To be completed by editor]

O! What a surprise.

Mark Carrington[1], Matthew K. Higgins[2]

[1] Department of Biochemistry, University of Cambridge

[2] Department of Biochemistry, University of Oxford

Standfirst

What evolutionary strategies are used by parasites to flourish for long periods within their mammalian hosts? These are questions which have been addressed by Pinger et al in a recent study which identifies variable O-glycosylation as a novel immune evasion mechanism employed by the African trypanosomes.

Main text

Hundreds of years ago the occupants of isolated alpine farms realised that if they banded together they could enhance their long-term survival in the face of marauding mercenaries who wandered into their valleys [1]. Subsequently, they enhanced this survival strategy by building hotels and converting visitors into pleasure-seeking tourists. Unanticipated bonuses for the farmers were that cuckoo clocks and cheese with holes went global, and the Swiss ended up as bankers to the world. This transition from short-term and probably violent interactions to long-term relationships is also central to the evolution of parasitism but the mechanisms that facilitate transmission and survival are also often unexpected.

Parasites are phylogenetically very diverse and parasitism has evolved countless times, so it is no surprise that they employ a huge range of mechanisms to allow survival in their hosts. However, a unifying property in the evolution of parasitism is a gain of the ability to negate the host's immune response, a pre-requisite for maintaining a long-term infection. The sexual cycle of many protozoan parasites of mammals occurs in the invertebrate vector, resulting in an evolutionary

imperative to survive for long enough to be ingested by a vector as it takes a blood meal. This has led to the evolution of various population survival strategies that avoid immune clearance of the infection rather than the individual, so that although the majority of individuals are killed, the infection persists. One route to this end is for antigenically novel clones to continually arise within the population and to expand before being killed by the adaptive immune response. Iterations of this process are known as antigenic variation.

The best characterised system of antigenic variation occurs in the African trypanosome, *Trypanosoma brucei,* which infects, and can cause disease in, most mammals including humans. A long history of detailed molecular analysis has shown that antigenic variation is dependent on a single class of protein, the variant surface glycoprotein VSG, which forms a coat covering the entire surface of the trypanosome cell [2]. The genome contains thousands of VSG sequences [3], and a monoallelic expression system ensures that only a single VSG is expressed at any one time. Antigenic variation occurs as a result of a switch in the identity of the expressed VSG that usually arises by a low frequency gene conversion of a new VSG into an active expression site. The genomic reservoir of VSGs in a single trypanosome genome is sufficient to allow decades of infection [4].

A comparison of the amino acid sequences of different VSGs showed that extreme sequence diversity underpins antigenic variation, with VSGs successively expressed in an infection typically showing less than 20% identity. As the VSG coat forms a shield that keeps host antibodies away from the plasma membrane [5], this raised the question of how such a diverse protein family can form a consistent barrier on the cell surface. Sequence analysis of a range of VSGs showed that they contain a mix and match of large N- and small C-terminal domains [6]. The N-terminal domains could be divided into three main types (A, B and C) based on the location of cysteine residues [7]. The structures of three type A N-terminal domains have been solved over the past 25 years [8 9] and reveal a dimeric organisation, with a remarkable conservation of structure despite extensive sequence diversity. It has been assumed that this conservation of structure is required to allow VSG to function as a shield.

In the intervening 25 years, the other VSG N-terminal domain types have remained in the shadows, with a general feeling amongst researchers that any differences from the structurally-characterised A-types would be minor. However, Pinger et al now report the structure of a VSG type B N-terminal domain from VSG3. Although this VSG is based on the same three helical bundle fold as the type A VSGs, its structure led to three unexpected discoveries.

First, VSG3 is monomer in solution, but packs into a trimeric arrangement at the higher concentrations of the crystal. Whether it is a monomer or a trimer on the cell surface remains to be determined, but the structure of VSG3 suggests that it does not form the homodimers predicted for all VSGs. This finding has consequences for our understanding of the organisation of the trypanosome surface. There is evidence that the distribution of trypanosome surface proteins on the cell surface is determined by the valency of the GPI-anchor, with dimeric VSG with two GPI anchors distributed across the whole cell surface, while receptors with one anchor are specifically localised to the flagellar pocket [10]. Whether VSG3 is monomeric on this cell surface, and so has a single anchor, need to be rationalised within this model.

Second, there a single O-linked glycosylation site located on the most membrane distal part of the VSG, on the surface most exposed to host immunoglobulins. The modification is heterogeneous, with between 0 and 3 hexoses, and O-linked glycosylation at a similar position in the structure was found on some other VSGs. This is the first demonstration of O-linked glycosylation in African trypanosomes.

Third, the O-linked oligosaccharide has a profound effect on virulence. Isogenic trypanosome lines expressing either VSG3 wild type or the same VSG with a point mutation S317A that abolishes O-linked glycosylated were used to compare virulence and antibody response. Cells expressing VSG3 S317A had reduced virulence in mice compared to the wild type. The cause of this reduced virulence was a result of both more effective IgM production against and a greater avidity of the IgM for VSG3 S317A. This indicates the O-glycosylation affects both IgM production and binding,

the latter possibly caused by the heterogeneity of the oligosaccharide. While the larger N-linked glycans are widely accepted to play an important role in reducing immunoglobulin binding to pathogen surface proteins, as seen in influenza haemogluttinin [11], it is fascinating to see their smaller cousin, when judiciously placed at the exposed tip of the VSG, playing a similar role. N-linked oligosaccharides in VSGs also display heterogeneity [5] and it may be time to revisit whether this also modulates the host immune response.

What are the consequences of these discoveries? First, they serve as another reminder that the evolution of parasitism will explore all possible sources of variation to outwit hosts. They also highlight that we still do not understand the interaction between immunoglobulins and the VSG coat. Indeed, our assumptions about the VSGs, based on sequence analysis, are a long way short of envisaging the depth of diversity that can be brought to the trypanosome surface by this one surface protein family. With the protozoan parasites dedicating hundreds or thousands of genes to avoid the host immune system, there are sure to be many more surprises to come even in the well-characterised African trypanosome.

Figure Legend

Figure 1 Representation of the origins of variation in VSGs

The genome contains thousands of VSG sequences. There are three or more N-terminal domain types and four or more C-terminal domains types and these can be mixed and matched during the gene conversion process central to antigenic variation [9]. Although N-and C-terminal domains are based on common folds there is surprising variation in structure. See Pinger et al. and the following PDBs: 1VSG, 2VSG, 5LY9, 6ELC, 1XU6, 2JWG, 2JWH and 5M4T. The trypanosome expresses a single VSG but heterogeneity within the coat is introduced by variation in glycosylation, in the O-linked oligosaccharide present on type B N-terminal domains (Pinger et al.), in the N-linked oligosaccharide(s) present on most VSG (reviewed in [5]) and in the galactosyl decoration of the core GPI-anchor [12]. The variation in O-linked oligosaccharide has a profound effect on the immune response of the host in the mouse model of infection.

References

1 Tuchman, B. W. *A distant mirror: the calamatous 14th century.*, (Albert A Knopf, 1978).

2 Horn, D. Antigenic variation in African trypanosomes. *Mol Biochem Parasitol* **195**, 123-129, doi:10.1016/j.molbiopara.2014.05.001 (2014).

3 Cross, G. A., Kim, H. S. & Wickstead, B. Capturing the variant surface glycoprotein repertoire (the VSGnome) of Trypanosoma brucei Lister 427. *Mol Biochem Parasitol* **195**, 59-73, doi:10.1016/j.molbiopara.2014.06.004 (2014).

4 Sudarshi, D. *et al.* Human African trypanosomiasis presenting at least 29 years after infection--what can this teach us about the pathogenesis and control of this neglected tropical disease? *PLoS Negl Trop Dis* **8**, e3349, doi:10.1371/journal.pntd.0003349 (2014).

5 Schwede, A., Macleod, O. J., MacGregor, P. & Carrington, M. How Does the VSG Coat of Bloodstream Form African Trypanosomes Interact with External Proteins? *PLoS Pathog* **11**, e1005259, doi:10.1371/journal.ppat.1005259 (2015).

6 Hutchinson, O. C. *et al.* VSG structure: similar N-terminal domains can form functional VSGs with different types of C-terminal domain. *Mol Biochem Parasitol* **130**, 127-131 (2003).

7 Carrington, M. *et al.* Variant specific glycoprotein of Trypanosoma brucei consists of two domains each having an independently conserved pattern of cysteine residues. *J Mol Biol* **221**, 823-835 (1991).

8 Blum, M. L. *et al.* A structural motif in the variant surface glycoproteins of Trypanosoma brucei. *Nature* **362**, 603-609, doi:10.1038/362603a0 (1993).

9 Bartossek, T. *et al.* Structural basis for the shielding function of the dynamic trypanosome variant surface glycoprotein coat. *Nat Microbiol* **2**, 1523-1532, doi:10.1038/s41564-017-0013-6 (2017).

10 Tiengwe, C., Bush, P. J. & Bangs, J. D. Controlling transferrin receptor trafficking with GPI-valence in bloodstream stage African trypanosomes. *PLoS Pathog* **13**, e1006366, doi:10.1371/journal.ppat.1006366 (2017).

11 Sun, X. *et al.* N-linked glycosylation of the hemagglutinin protein influences virulence and antigenicity of the 1918 pandemic and seasonal H1N1 influenza A viruses. *J Virol* **87**, 8756-8766, doi:10.1128/JVI.00593-13 (2013).

12 Izquierdo, L., Acosta-Serrano, A., Mehlert, A. & Ferguson, M. A. Identification of a glycosylphosphatidylinositol anchor-modifying beta1-3 galactosyltransferase in Trypanosoma brucei. *Glycobiology* **25**, 438-447, doi:10.1093/glycob/cwu131 (2015).