Maintaining the protective variant surface glycoprotein coat of African trypanosomes

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Abstract

The African trypanosome *Trypanosoma brucei* has a precarious existence as an extracellular parasite of the mammalian bloodstream, where it is faced with continuous immune attack. Key to survival is a dense VSG (variant surface glycoprotein) coat, which is repeatedly switched during the course of a chronic infection. New data demonstrate a link between VSG synthesis and cell cycle progression, indicating that VSG is monitored during the trypanosome cell cycle.

The structure of the protective VSG (variant surface glycoprotein) coat

Bloodstream form *Trypanosoma brucei* is coated with a densely packed layer of VSG, which is attached to the surface membrane via a GPI (glycosylphosphatidylinositol) anchor. An intact VSG coat protects the trypanosome from lysis by the alternative pathway of the complement system [1,2]. However, VSGs are highly antigenic, and eventually the trypanosome succumbs to antibody-mediated lysis. The parasite therefore relies on its ability to switch between the mutually exclusive expression of one of hundreds of immunologically distinct VSGs to maintain a chronic infection [3].

VSGs have a highly conserved tertiary structure with an extremely diverse N-terminal 'variable' domain containing two long antiparallel α-helices separated by a turn [4]. Insertion of epitope tags into various regions of VSG can result in drastic reductions in expression level, indicating stringent quality controls on these molecules before they reach the parasite surface [5]. A striking feature of VSGs is that regions of the molecule which are highly divergent (only 16% conservation in amino acid sequence), can nonetheless have very similar tertiary structures [4,6]. The *VSG* gene family therefore appears to have evolved through a 'tug of war' between two evolutionary selection pressures: one for antigenic diversity resulting in sequence divergence, and another for conservation of tertiary structure, which presumably facilitates packing of different VSGs within the coat of a switching trypanosome [7]. The VSG layer shields invariant proteins on the cell surface from recognition by antibodies. The VSG-fold (a conserved tertiary structure characteristic of VSG) can also be found in a variety of invariant surface molecules, presumably allowing a non-disruptive fit into the protective VSG layer [7–9].

Key words: antigenic variation, immune evasion, RNA interference (RNAi), *Trypanosoma brucei*, variant surface glycoprotein, *VSG* expression site.

Abbreviations used: GPI, glycosylphosphatidylinositol; IgM, immunoglobulin M; RNAi, RNA interference; VSG, variant surface glycoprotein.

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The 'fluid' VSG coat and immune evasion

The VSG coat is a highly fluid surface barrier, with rapid lateral diffusion of GPI-anchored VSG molecules within the coat [10]. There is no evidence for significant lateral protein–protein interactions between the VSG dimers, or for self-assembly of VSG dimers into larger two-dimensional assemblies [11]. Trypanosome clearance in immunized animals appears to be primarily mediated by a T-cell independent IgM (immunoglobulin M) response [12,13]. It is likely that the specific nature of the VSG surface coat architecture, with its repetitive arrays of closely packed identical VSG homodimers plays a critical role in triggering T-cell independent B-cell responses [13,14].

Although trypanosomes are very effectively lysed in the presence of anti-VSG IgG or IgM, they are unaffected by low antibody titres owing to their ability to remove anti-VSG antibodies from the cell surface through endocytosis [15]. Bloodstream form *T. brucei* has one of the highest rates of endocytosis measured, resulting in the complete turnover of the entire pool of surface VSG every 12 min [16]. These extremely high rates of endocytosis could function as a 'coat cleaning machine', stripping host molecules off of the trypanosome surface, and shunting complexes of VSG covalently bound to host molecules for degradation in the lysosome. This would make high rates of endocytosis a critical protective adaptation, increasing the pathogenicity of the bloodstream form of trypanosome [17].

The link between VSG and the trypanosome cell cycle

In order to investigate the role of VSG and determine whether we could make 'naked' bloodstream form trypanosomes, we performed VSG RNAi (RNA interference) *in vitro* [18]. Induction of RNAi against the active VSG results in very rapid ablation of VSG transcript within 4 h. This triggers a rapid and specific cell-cycle arrest, whereby cells stall precytokinesis after completion of mitosis, but before initiation of a cleavage furrow. There is no evidence for reinitiation of S phase within these arrested cells.

This phenotype is particularly striking in comparison with other precytokinesis blocks that have been described in bloodstream form *T. brucei*. Mottram and co-workers inhibited *T. brucei* homologues of Mob1, which is required for cytokinesis in *S. pombe* [19]. After the induction of Mob1 RNAi in bloodstream form *T. brucei*, a delay in cytokinesis is observed. The majority of the cells have a detectable cleavage furrow, however there is an accumulation of cells with multiple nuclei indicating reinitiation of S phase. Similarly, inhibition of synthesis of GPI8, which is involved in the addition of the preformed GPI anchor on to nascent polypeptides, results in a precytokinesis block [20]. However, these cells are only partially blocked within the cell cycle, as 'monster' cells with multiple flagella and nuclei accumulate within the culture. The VSG RNAi induced arrest is the first precise precytokinesis arrest described in bloodstream form *T. brucei*. This argues that VSG transcript or protein is monitored during the cell-cycle, and that a specific checkpoint is triggered in the absence of either VSG synthesis or VSG on the cell surface.

Consequences of VSG restriction

Surprisingly, the total amount of VSG in these VSG RNAi stalled trypanosomes did not appear visibly decreased using immunofluorescence microscopy or Western-blot analysis, even after a block in VSG synthesis for 24 h [18]. VSG has an unusually long half-life (more than 33 ± 9 h), allowing stalled cells to persist in the absence of VSG synthesis [21]. Cells stalled after the induction of VSG RNAi for 24 h were shorter and broader than normal, although there was no visible reduction in cell volume. This morphology could be a consequence of VSG restriction resulting in the cell attempting to minimize its surface area-to-volume ratio. The short and broad morphology is superficially similar to that of 'stumpy' form parasites, which are non-dividing forms arising at high parasite densities [22]. In these 'stumpy' cells, VSG synthesis has also ceased [23], suggesting that the 'stumpy' morphology could be a consequence of VSG restriction.

Does this cell-cycle arrest triggered in the absence of VSG synthesis indeed have a protective function for the trypanosome? Induction of VSG RNAi *in vivo* leads to very rapid clearance, arguing that minor chinks might develop in the protective coat, which are exploited by an immunocompetent animal. This indicates the essentiality of a completely intact VSG coat for immune evasion. Although blocking cell division in the absence of VSG synthesis prevents drastic dilution of the VSG coat, this arrest is not adequate to protect the trypanosome in immunocompetent animals. Our experiments result in very rapid ablation of VSG transcript down to 1–2% of normal levels within 8 h. Possibly we have overwhelmed a putative protective mechanism, and the VSG 'sensing' mechanism linking VSG synthesis to progression through the cell-cycle has evolved to absorb less drastic fluctuations in VSG synthesis.

Clearly, it will be important to determine what aspect of VSG or its synthesis is 'monitored' in order to trigger the cell-cycle arrest. What processes have stopped in these arrested cells? Is there continued synthesis of GPI anchors? What exactly is the mechanism whereby these cells stalled by VSG RNAi are cleared in mice? The extreme sensitivity of trypanosomes to inhibition of VSG synthesis *in vivo* argues that chemicals that perturb VSG synthesis or processing would be promising lead drug candidates for trypanosomiasis.

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