# VSG overcomes an early barrier to survival of African trypanosomes in tsetse flies

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The widespread expansion of vector-borne diseases is a testament to their success. According to the World Health Organization, over half the global population is at risk for contracting a vector-borne disease, and over a million deaths are annually attributed to diseases transmitted by insects (1). An absence of preventative vaccines combined with the rising resistance to insecticides has led to a surge in efforts to develop alternate approaches toward vector control. One such approach is the interruption of pathogen transmission. Understanding the molecular basis of parasite–vector interactions can identify critical steps in pathogen development to be targeted for disruption. After millions of years adapting to their vectors, pathogens have evolved complex and innovative survival strategies aimed at overcoming host defenses. For example, both *Leishmania* parasites and *Borrelia* spirochetes use abundantly expressed surface proteins to specifically bind receptors on epithelial cells, promoting successful gut colonization (2, 3). Importantly, blocking these receptors effectively disrupts the establishment of *Leishmania* and *Borrelia* in their respective sand fly and tick vectors. Another attractive target for interruption of pathogen transmission is manipulation of the peritrophic matrix (PM). Synthesis of a PM is a common response to blood feeding and has been adopted by several disease vectors (4–7). This chitinous network of proteins acts as a protective barrier that isolates midgut epithelial cells from harmful substances and pathogens ingested with the blood. Upon completion of bloodmeal digestion, the PM is egested together with any remaining contents. To overcome this early barrier to development, some parasites—such as *Plasmodium* and *Leishmania*—secrete chitinases, facilitating their escape through the PM (8, 9). Additionally, *Leishmania* parasites take advantage of the vector sand fly chitinase to aid its escape (10). Relevantly, silencing the sand fly chitinase compromises *Leishmania* midgut development (11); additionally, inhibiting the activity of the *Plasmodium* chitinase blocks oocyst development (12). In tsetse, integrity of the PM is also vital to containment of trypanosome infections, and the immature PM of young teneral flies renders them more susceptible to infection compared with adults that produce a mature PM (13). Importantly, the mechanism by which trypanosomes traverse the PM to enable gut colonization in susceptible tsetse had been unknown until now. In PNAS, Aksoy et al. (14) share their discovery of how trypanosomes disrupt the PM of the tsetse fly and implicate the variant surface glycoprotein (VSG) of the blood stream form (BSF)—famed for its critical part in escaping the immune system of the mammalian host (15)—in its disruption, revealing a dual role for VSG in the life cycle of trypanosomes.

Infected blood ingested by a tsetse fly contains a large number of slender and a few stumpy BSF trypanosomes, both expressing a similar coat of VSG molecules (5). Slender trypanosomes cannot survive in the vector and are lysed, and stumpy forms differentiate within hours to procyclic forms, shedding their VSG coat and replacing it with procyclin (5). At this time, the parasites are still confined within a type 2 PM secreted in a continuous manner by cells of the cardia, a specialized tissue at the junction of the midgut and the foregut (mouthparts) of the tsetse. To continue their development in the vector, procyclic trypanosomes need to escape into the ectoperitrophic space and colonize the gut. In PNAS, Aksoy et al. (14) demonstrate that VSG molecules, abundantly present in the gut lumen as a by-product of lysed slender forms or after shedding by stumpy forms, are not squandered but are used to disrupt the structural and functional integrity of the PM, ensuring continuation of the parasite's life cycle (Fig. 1). Using RNA sequencing, the authors first determined gene expression in midgut cells at 48 and 72 h after feeding mature adults on trypanosome-infected or normal blood. Aksoy et al. found that trypanosomes reduced the expression of peritrophins, structural proteins that bind chitin, and digestive enzymes, both associated with PM formation in insects (16–18). Then Aksoy et al. (14) used the entomopathogenic *Serratia marcescens* in an elegant microbial assay to assess the PM integrity in tsetse flies. If *Serratia* is protected by a functional PM, it multiplies and eventually kills the flies.

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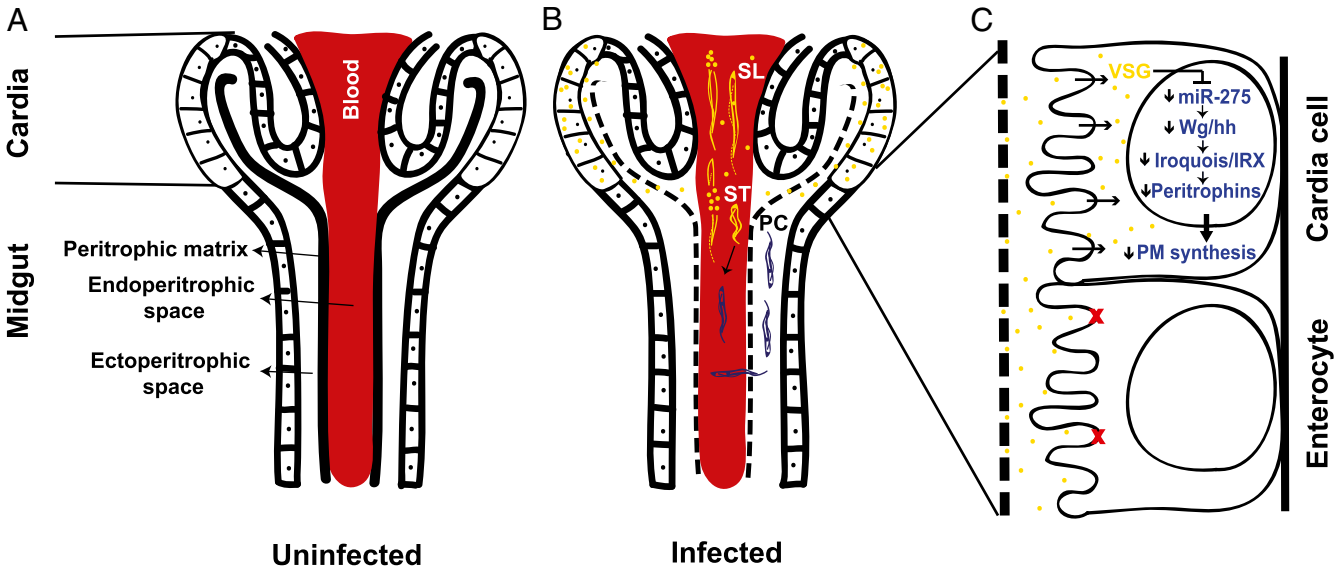


**Fig. 1. VSG-mediated disruption of the peritrophic matrix in tsetse flies. (*A* and *B*) Schematic representation of the anterior portion of the tsetse midgut depicting the cardia, midgut cells (enterocytes), and PM. The PM compartmentalizes the midgut lumen into an ectoperitrophic and an endoperitrophic space. (*A*) A functional PM (solid line) surrounding an uninfected bloodmeal. (*B*) A disrupted PM (dashed line) surrounding a trypanosome-infected bloodmeal. Cells of the cardia internalize VSG (yellow dot) made available by lysis of slender (SL) forms, or after shedding by stumpy (ST) forms as they differentiate into procyclics (PC) that cross the compromised PM. (*C*) Close-up of a cardia cell and an enterocyte depicting the specific uptake of VSG by cardia cells. VSG uptake leads to down-regulation of mi*R*-275 that reduces expression of the Wingless (wg) and Hedgehog (hh) signaling pathways and the Iroquois/IRX family of transcription factors. This leads to a reduction in peritrophins and a disrupted PM.**

Conversely, if the microbes leak out of a disrupted PM they activate immunoreactive epithelial cells and are rapidly killed, and the tsetse flies survive. As expected, when the tsetse flies were provided BSF extracts and *Serratia*, they survived significantly better than controls. However, procyclic extracts had no effect on fly survival, indicating that only BSF extracts disrupted the function of the PM, increasing its permeability. To understand how BSF trypanosomes compromise the production of PM-associated proteins, the authors deep-sequenced small RNAs from the same material used for gene-expression analysis of midgut cells at 72 h. Aksoy et al. note the decreased expression of several regulatory microRNAs, among them *miR*-275, in trypansosome-infected compared with normal blood-fed guts. *miR*-275 was of particular interest to the authors because of its increased expression in cardia compared with midgut tissue. Moreover, *miR*-275 has been associated with gut function and digestion in mosquitoes (19). Upon neutralization of the function of *miR*-275 using an antisense oligonucleotide (ant-275), the expression of the Wnt signaling pathway and the Iroquois/IRX-family of transcription factors—established by Aksoy et al. (14) as cardia-specific regulatory molecules in tsetse flies that govern the synthesis of the PM—as well as the expression of peritrophins, was inhibited in cardia tissue. Additionally, blood digestion and diuresis were delayed. Similarly, using gene-specific small-inhibitory RNAs targeting molecules in the Wnt signaling pathway, the expression of both the Iroquois/IRX-family of transcription factors and peritrophins was again reduced. Taken together, these data provide strong evidence that *miR*-275 regulated the synthesis of the PM by modulating the Wnt signaling pathway and the Iroquois/IRX-family of transcription factors, alluding to the mechanism by which trypanosomes escape the tsetse PM.

However, what molecule in BSF compromises the structure of the PM via the regulatory *miR*-275? To address this important question, Aksoy et al. (14) used the microbial assay with *Serratia* to test the most abundant surface molecule of BSF, VSG. The authors show that provision of soluble VSG (sVSG) in the bloodmeal promoted survival of tsetse flies, similar to BSF extracts. Additionally, flies given sVSG had higher weights and increased hemoglobin, indicative of an irregularity in blood digestion. Next, the authors demonstrate that sVSG was actually internalized by cells of the cardia, where it reduced the expression of *miR*-275 and the associated Wnt signaling pathway and the Iroquois/IRX-family of transcription factors, and produced a functionally compromised PM (Fig. 1). The compromised PM also led to a higher susceptibility to trypanosome infection; this was demonstrated by feeding mature flies sVSG followed by BSF trypanosomes in the subsequent bloodmeal, or feeding teneral flies procyclics together with sVSG. Both mature and teneral flies showed a significantly increased susceptibility to infection, although the former required a higher amount of sVSG (10 μg/mL vs. 1 μg/mL). It is noteworthy that the compromised PM and digestive processes had no long-term effect on survival and fitness of the flies, which points to a refined evolutionary adaptation, whereby VSG transiently compromises the PM, opening a short window of opportunity for early gut colonization by trypanosomes while ensuring there is no long-term damage to the insect host.

Although VSGs from various strains tested by Aksoy et al. (14) resulted in a comparable effect on the PM, the authors advocate a wide-range investigation of field isolates to assess the effect, if any, of changes in side chains of VSG, particularly those adapted to cattle versus humans. Such studies may reveal clues as to why a proportion of mature field tsetse flies are susceptible to infection and why, intriguingly, the prevalence of infection in nature actually increases with age of the flies. Moreover, unraveling the mechanism by which VSG is specifically internalized by cells of the cardia can potentially shed light on how to block an event critical to trypanosome survival in the gut. Although it is well recognized that susceptibility to infection is multifactorial, influenced not only by age of the fly but also by other factors, such as

the composition of its gut microbiome (13, 20), it remains that interruption of trypanosome development in the tsetse at such an early step in gut colonization represents an attractive target for parasite control.

The work by Aksoy et al. (14) represents a major advancement in our understanding of tsetse–trypanosome molecular interactions, revealing how the parasites migrate from the gut lumen into the ectoperitrophic space. This naturally begs the question: How would the epimastigote trypanosome reenter the endoperitrophic space? Another mystery in the parasite's life cycle within the tsetse; this step is required to access salivary ducts or glands where metacyclogenesis occurs. Importantly, how trypanosomes traverse the PM in both directions represents merely two of the many steps along the long ladder of vector–parasite interactions, underscoring their untapped potential as targets of interventions to interrupt transmission.

The changing repertoire of the VSG coat has been a major impediment to the development of human vaccines against trypanosomiasis. It is therefore understandable why emphasis has been placed upon the study of VSG in the mammalian host. In PNAS, Aksoy et al. (14) provide evidence of a novel and critical role for VSG in the insect vector, where it ensures survival of procyclic parasites past bloodmeal digestion. At this early stage, the parasites, likely still few in number, may be more vulnerable to elimination. Now, we can add VSG to our arsenal of molecules that can be targeted for control of African trypanosomes, not only in the human host but in the tsetse vector as well.


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