VSGs: "you'll never express alone". MISP, a family of metacyclic invariant surface proteins in trypanosomes.

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The development of *Trypanosoma brucei* within the tsetse vector is accompanied by the expression of several stage-specific families of GPI-anchored surface glycoproteins. We recently discovered that saliva from *T. brucei*-infected tsetse flies is enriched with Brucei Alanine-Rich Proteins (BARP), VSG and a novel family of GPI-anchored surface glycoproteins. The latter are phylogenetically grouped within the Clade IV of family 50 of trypanosome surface proteins and are encoded by five paralogs, whose products are over 90% identical in sequence. Immunofluorescence and transcript analysis showed that Clade IV proteins are expressed on the surface of metacyclic trypanosomes and also on epimastigotes and pre-metacyclic forms although in lower abundance. This expression pattern opposes that of BARP, which is highly expressed in the epimastigote stage and diminishes during differentiation to metacyclics. Because Clade IV proteins are almost identical in sequence and are heavily expressed in the metacyclic stage, we named them Metacyclic Invariant Surface Proteins (MISP). In order to gain insights into the function of MISP proteins, we expressed isoform MISP.360 and determined its crystal structure at1.8 Å resolution. MISP.360 adopts an extended helical bundle structure with an overall shape that highly resembles that of VSG and BARP despite their high degree of sequence divergence. Furthermore, molecular modeling studies suggest that MISP proteins are projected on top of the metacyclic VSG coat. We postulate that MISP might be important 1) to maintain the tight intermolecular packing with VSG molecules on the metacyclic surface, and 2) for parasite development in the tsetse salivary glands.