TSSA: from yet another *Trypanosoma cruzi* surface antigen to the most appealing candidate for the development of parasite serotyping assays

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Trypanosoma cruzi, the protozoan agent of Chagas Disease, displays a highly structured population, with multiple strains that could be grouped into six discrete typing units (DTU). In addition to extensive genetic variability, these DTUs present distinct geographical distribution, specific ecological associations with (at least partially) non-overlapping spectra of competent vectors and/or susceptible mammals, and differences in relevant epidemiological/clinical traits such as vector infectivity, vector-to-mammal transmissibility, susceptibility to drugs and parasitemia. In this framework, methods able to assign the *T. cruzi* infecting strain type directly in blood samples are expected to have a positive effect on both parasite epidemiologic surveillance and clinical management of Chagasic patients. Serological typing assays that exploit the presence of strain-specific antibody signatures to polymorphic *T. cruzi* antigens emerge as an appealing approach to address this issue. TSSA (Trypomastigote small surface antigen) was originally identified as 'yet another parasite antigen', this one encoded by an apparently single-copy, mucin-like gene expressed by bloodstream trypomastigote forms. Sequencing of TSSA alleles from different strains, however, revealed several polymorphisms that defined 4 main protein variants, each one corresponding to an ancestral DTU (TcI to TcIV). Interestingly, TSSA variants were shown to display major antigenic differences, hence pointing to this molecule as a promising *T. cruzi* serotyping candidate. Indeed, epidemiological, and clinical surveys conducted so far have shown that, despite certain aspects that need to be improved, TSSA is able to provide robust, sensitive, low-cost, and point-of-sampling diagnosis, with near DTU-level resolution. The advent of high-quality parasite genomes showed a larger-than-expected complexity of TSSA sequences. In addition to variations in TSSA gene dosage among *T. cruzi* strains, our recent genome mining exercises revealed the presence of TSSA pseudogenes and two TSSA hemizygous loci in hybrid DTUs (TcV and TcVI). Most relevant, they allowed the identification of several novel TSSA variants and one TSSA sequence from the phylogenetically related bat parasite *T. cruzi marinkellei*. Despite its evident genetic drift, this molecule shared quite similar structural features with *T. cruzi* TSSAs, thus raising doubts about its evolutionary origin and distribution and, most importantly, warranting further investigations on the diagnostic impact of such atypical variants. Overall, our data shed new light into TSSA evolution, diversity, and antigenic landscape, and contribute to improving the design, resolution, and specificity of Chagas Disease diagnostic applications, and particularly of *T. cruzi* serotyping strategies.