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Trends in Parasitology

Spotlight

Single-cell transcriptomics reveals hidden information in trypanosomatids

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Dixenic parasites often encounter environmental extremes during the transition from vector to host. Preadapted transmission stages overcome these challenges to promote parasites' survival and ensure life cycle progression. Recently, Vigneron *et al*. and Briggs *et al*. used single-cell transcriptomics to investigate developmental stage specific gene expression patterns during parasite differentiation.

Trypanosoma brucei, which causes the neglected diseases human and animal African trypanosomiasis, has a complex developmental life cycle, transiting between invertebrate (tsetse fly) and vertebrate (mammalian) hosts. Upon entry, parasites are confronted with species-specific environments and encounter numerous challenges, such as variations in nutrient availability and immune defences. To successfully establish an infection, *T. brucei* differentiates before transmission into preadapted life cycle stages primed for survival in these harsh environments [1].

T. brucei lives extracellularly in the blood and tissue fluids of a mammalian host as long 'slender' bloodstream forms (BSFs), and within the fly midgut as procyclic forms (PCFs). These life cycle stages are well studied. However, intermediate life cycle stages, and transmissible forms, which contribute to the spread of the disease, are less understood despite representing key stages in the life cycle. *T. brucei* has two transmissible forms, both of which are nonreplicative. Increased blood parasitaemia triggers the differentiation of BSFs into short 'stumpy' forms via quorum sensing [2] whereas, in the fly, PCFs differentiate to epimastigotes and further into mammalian infective metacyclic forms that reside within the tsetse's salivary glands. Although population-based transcriptomic studies have contributed to our understanding of the two transmission stages, their precision is limited. Bulk transcriptomics lacks the resolution to examine less prominent or transient changes to the transcriptome during differentiation, which occurs asynchronously. Furthermore, these intermediate alterations can be lost during data normalization. Now, in the era of single-cell genomics, single-cell transcriptomics (scRNA-seq) studies can overcome these limitations [3]. Two recent studies – by Vigneron *et al*. [4] and Briggs *et al*. [5] – used Chromium Single-Cell technology from 10x Genomics, to precisely profile differential gene expression patterns associated with *T. brucei* differentiation, constructing temporal trajectories of metacyclogenesis and the slender-to-stumpy transition.

Vigneron *et al*. [4] examined transcript changes during the process of metacyclogenesis from a pool of 2045 parasites extracted from infected tsetse salivary glands. The authors described the presence of three distinct transcriptional clusters, epimastigotes (Epim), premetacyclics (Meta1), and mature metacyclics (Meta2). They supported their clustering using prior described alterations to parasite metabolism, which shifts from amino-acid-based in the vector (Epim) to glycolysis in the vertebrate host (Meta1 and Meta2), and to surface-expressed genes. They reported differential expression of known factors promoting metacyclogenesis, including RNA-binding proteins (RBPs), calflagins, and zinc-finger-binding proteins. For instance, they detected a peak of enhanced RBP6 transcription during the epimastigote-to-metacyclic transition, and increased transcription of RBP10 in mature metacyclic cells. RBP10 plays a key role in mediating the metacyclic-to-BSF transition [6]. Interestingly, mature metacyclics (Meta2) were the most transcriptionally active cluster, suggesting that the parasites may enhance their transcriptional capacity in preparation for a rapid differentiation into BSFs once transmitted to the host. Although premetacyclics were less transcriptionally active, the enrichment of factors putatively associated with microtubule movement and proteolysis implies that the parasites may prioritise rearranging their cellular structure, from epimastigote to metacyclic-like, prior to metabolic alterations. In support, as the parasites transition to the mature and infectious metacyclics (Meta2), enriched expression of genes associated with glucose-based metabolism occurs. Additional subclustering uncovered a putative cluster of 'intermediate' forms (Inter) between the epimastigote and premetacyclic stages, although the reduction in RBP6 expression and the increasing expression of metacyclic variant surface glycoproteins (VSGs) suggests that this cluster may reflect premetacyclic cells during their early stages of differentiation. In all, the authors validate scRNA-seq as a powerful technique for profiling parasite differentiation and additionally uncover key transcriptional changes occurring during this process.

Another important finding that emerged from this study was the enrichment of a family of nonvariant surface proteins (Fam10) within the metacyclic clusters. The roles of Fam10 proteins are still unknown. However, invariant surface proteins on the surfaces of extracellular pathogens can be promising vaccine candidates. Recently, immunity to infection

acquired through vaccination with an invariant surface protein has been demonstrated in *Trypanosoma vivax* [7]. Thus, limiting disease progression by targeting the transmissible life cycle stage in *T. brucei* could overcome the parasite's extreme capacity to circumvent the host adaptive immune response. Vigneron *et al*. [4] vaccinated mice using a recombinant member of the Fam10 protein family, SGM1.7. Following immunization, they reported reduced parasite survival during the initial stages of infection. They reported similar findings using another Fam10 family member (SGM1.3) (Figure 1). Altogether, the authors demonstrated that metacyclogenesis is a gradual adaptation process, likely to also ensure continued persistence of the parasites within the fly.

In the second study, Briggs *et al*. [5] modelled the slender-to-stumpy transition using oligopeptides, which stimulates *in vitro* differentiation irrespective of population density [8]. Using this approach, the authors performed scRNA-seq across a pool of cells harvested at varying times after exposure to oligopeptides, capturing a range of 'differentiation' states across 8599 parasites.

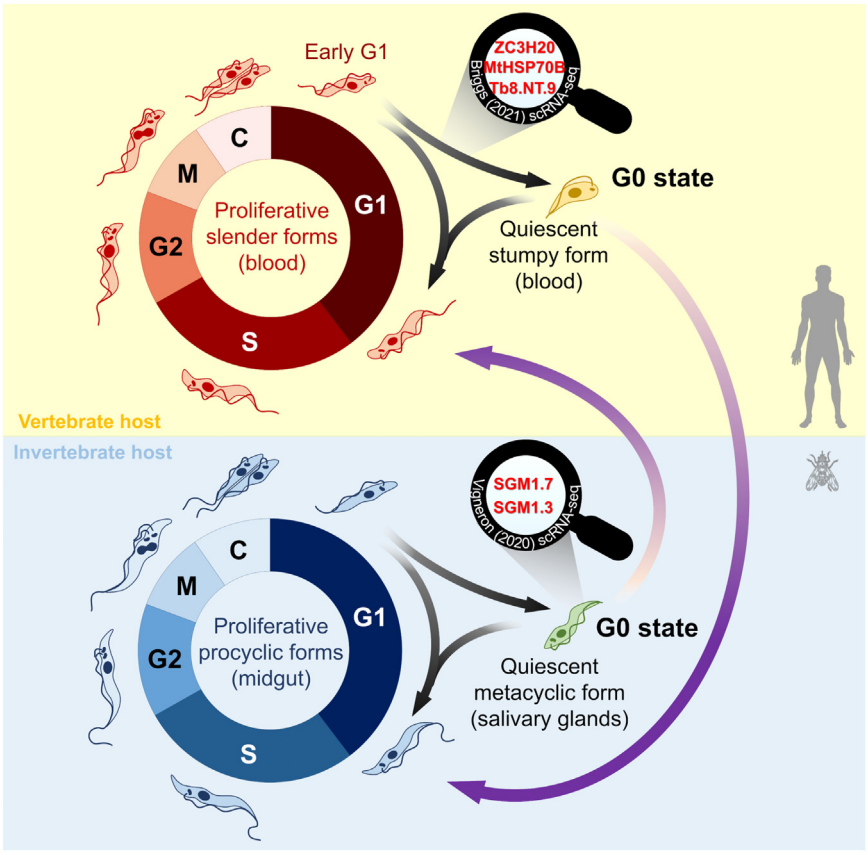

Figure 1. Single-cell transcriptomics uncovers known and putative regulators of *Trypanosoma brucei* differentiation. Briggs *et al.* [5] revealed that the differentiation toward transmissible forms (from proliferative slender to quiescent stumpy in vertebrate hosts) is regulated by key factors (e.g., ZC3H20, MtHSP70B, Tb8.NT.9, among others), further showing that quiescent stumpy forms exit the cell cycle during early G1 phase. Vigneron *et al.* [4] analysed the transcriptome of *T. brucei* transition forms (from proliferative procyclic to infective metacyclic), isolated from tsetse salivary glands. The authors identified a family of nonvariant surface proteins (Fam10) associated with the metacyclics. Some members of this family (e.g., SGM1.7 and SGM1.3) exhibited potential as transmission-blocking vaccine antigens. The purple arrows highlight the transition between invertebrate (blue background) and vertebrate (yellow background) hosts.

The authors defined four distinct transcriptional forms (SlenderA, SlenderB, StumpyA, and StumpyB). However, no distinct 'intermediate' stage between slender and stumpy was reported. Over 60% of the identified genes were previously described as differentially expressed between slender and stumpy cells [9]. Furthermore, they found an additional ~600 hypothetical genes associated with this transition. The authors next resolved cell-cycle-associated gene expression changes in their replicative slender-cell populations. By comparing transcriptomic changes captured from bulk RNA-seq in *T. brucei*, they defined clusters of cells reflecting G1, S, or G2/M phases, uncovering novel putative cell-cycle-regulated factors. Additionally, the cell-cycle expression profile uncovered the possible point of cell-cycle exit taken by 'stumpy' forms that are nonreplicative (G0 state). This transition likely occurs during the early G1 phase. To investigate this point of stumpy commitment further in slender cells, they monitored gene expression patterns via scRNA-seq in parasites lacking the ZC3H20 protein. ZC3H20 peaks in expression during the slender-to-stumpy transition and has been previously shown to drive differentiation *in vivo* and *in vitro* to stumpy cells [10]. Intriguingly, Vigneron *et al*. [4] report a peak of ZC3H20 expression in early premetacyclic stages, perhaps also indicating a role for ZC3H20 during the commitment to metacyclogenesis. In all, this approach identified putative early regulators of commitment during the slender-to-stumpy transition, which include more than two dozen putative genes to be prioritised for further studies (Figure 1).

Despite tacking two extreme stages in its life cycle, striking parallels can be drawn

between the two ways of progression to the transmissible (stumpy/metacyclic) *T. brucei* forms: (i) this process appears to be progressive, with limited or indeterminate 'intermediate' stages; (ii) differential expression of factors, such as RBPs and chromatin remodelling proteins, suggests that, although gene expression in trypanosomatids is largely considered post-transcriptional, regulation at the genomic and transcriptional levels are prominent, playing a pivotal role in parasite differentiation; (iii) the 'decision' to commit to a virulent form likely triggers early in the cell cycle (G0 state), perhaps because commitment with transmission (infection) is mutually exclusive relative to the commitment with cell division.

Further studies utilising scRNA-seq in *T. brucei* are emerging. However, one such study is yet to address, with precision, the transition between metacyclic to slender BSF. Though experimentally challenging, understanding what gene expression changes drive this differentiation into the disease-causing stage will be vital in halting disease development in the mammalian host.

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Declaration of interests

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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