A major step towards defining the elusive stumpy inducing factor in *Trypanosoma brucei*

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Abstract

Trypanosoma brucei stumpy forms are the only stage that can transmit from human to tsetse fly. Stumpy formation is regulated by a quorum sensing mechanism that depends on parasite density and an unknown stumpy induction factor (SIF). Recently, an elegant study by Matthews and colleagues has identified several crucial components of this pathway including the putative SIF and its receptor.

The protozoan parasite *Trypanosoma brucei* causes sleeping sickness in humans and nagana in animals in sub-Saharan Africa. Trypanosomes cycle between a mammalian host and an arthropod vector, the tsetse fly, thereby transitioning through various environments. Importantly a subset of parasites switches from a replicative, slender form in the human blood stream to a non-replicative stumpy form that is competent for transmission to the tsetse fly. Stumpy forms undergo drastic morphologic and metabolic changes in preparation for further development in the tsetse fly's midgut.

Proposed more than 20 years ago, various lines of evidence have established the paradigm that quorum sensing regulates the level of stumpy formation. In this model slender forms have the capacity to sense their density in the host (and *in vitro*) based on the abundance of a soluble factor termed stumpy induction factor (SIF) [1]. Earlier experiments also demonstrated that SIF is transduced in the parasite through cyclic AMP-dependent signalling [2]. A genome-wide RNAi screen has recently identified more than 30 activators and repressors of the SIF pathway, finally providing the basis for a systematic dissection of the downstream factors required for stumpy formation [3]. A series of subsequent studies have started to validate several of these factors, including the serine/threonine phosphatase PPT1 and the RNA binding protein RBP7 and placed them in a non-linear hierarchy of the SIF pathway [4]. So far, the nature of SIF remained elusive.

A recent study by Matthews and colleagues represents a major step towards defining the SIF pathway. In their study the *T. brucei* ortholog of the mammalian G protein coupled-receptor (GPCR) protein GPR89 is identified as an essential surface antigen required for parasite survival and stumpy formation. Inspection of the protein revealed that TbGPR89 has both a

GPCR and an oligopeptide transporter domain (with homology to bacterial proton-coupled peptide transporters POT), suggesting that it has a dual function as a receptor and transporter. In contrast, other Trypanosoma species lacking density-dependent growth control have two separate proteins, a POT and a GPR89 ortholog. Trypanosomes are known to secrete peptidases into their environment during infection, generating unusual oligopeptides in the blood stream [5]. Indeed, analysis of the secretome of *T. brucei* identified multiple secreted peptidases [6], with proposed function in modulating host immunity and pathogenesis [7]. The new study by Rojas *et al.* demonstrates that TbGPR89 is an oligotransporter for oligopeptide substrates that are produced by secreted peptidases. Importantly, inducible expression of such secreted peptidases can induce stumpy formation. Altogether these data demonstrate that secreted peptidases can generate a paracrine quorum sensing signal in the form of oligopeptides that is internalised by TbGRP89 and can subsequently lead to stumpy formation, recapitulating all the hallmarks of SIF.

The study by Rojas *et al*. represents a major breakthrough towards defining the elusive SIF and its receptor, and as such it closes a critical knowledge gap in the parasite cycle. The authors propose a model where levels of secreted peptidases (essentially a proxy for parasitemia) determine the concentration of oligopeptides in the parasite environment. These oligopeptides are taken up by recipient cells via TbGRP89, and once they reach a certain threshold induce stumpy differentiation in recipient cells (Figure 1). However, many open questions remain that will need to be addressed in future studies. First, it is not clear whether parasites are sensitive to the oligopeptide concentration or the type of oligopeptides they are exposed to (or a combination thereof). Second, experiments were performed under *in vitro* conditions and in the mouse model where a significantly higher parasitemia is reached than

during human infection. Therefore, a localized rather than systemic concentration of oligopeptides is more likely to be critical under physiological conditions. Recent studies have demonstrated parasite homing to skin [8] and adipose tissues [9] where stumpy formation is promoted. Given the new findings, this may be the result of increased local concentration of oligopeptides in these microenvironments. Such a scenario is similar to the recently described regulation of gametocyte formation in the malaria parasite, *Plasmodium falciparum* via the serum phospholipid lysophosphatidylcholine (LysoPC) [10]. While reduced systemic levels of LysoPC are mainly induced by inflammation (hence a putative trigger of differentiation) they are intrinsically lower in the bone marrow niche, where gametocyte levels are high. Third, it is unclear how TbGRP89 itself is regulated to fine tune its dual function as transporter and sensor. Investigation of the functional domains of TbGPR89 identified putative phosphorylation and glycosylation sites that could regulate its activity, possibly via the known SIF signalling factors PP1 and MEKK1. Finally, this important study opens new avenues for drug development: as TbGRP89 is essential both for parasite survival and stumpy formation, a Tb-GRP89-targeting drug could potentially reduce parasite burden and at the same time block transmission.

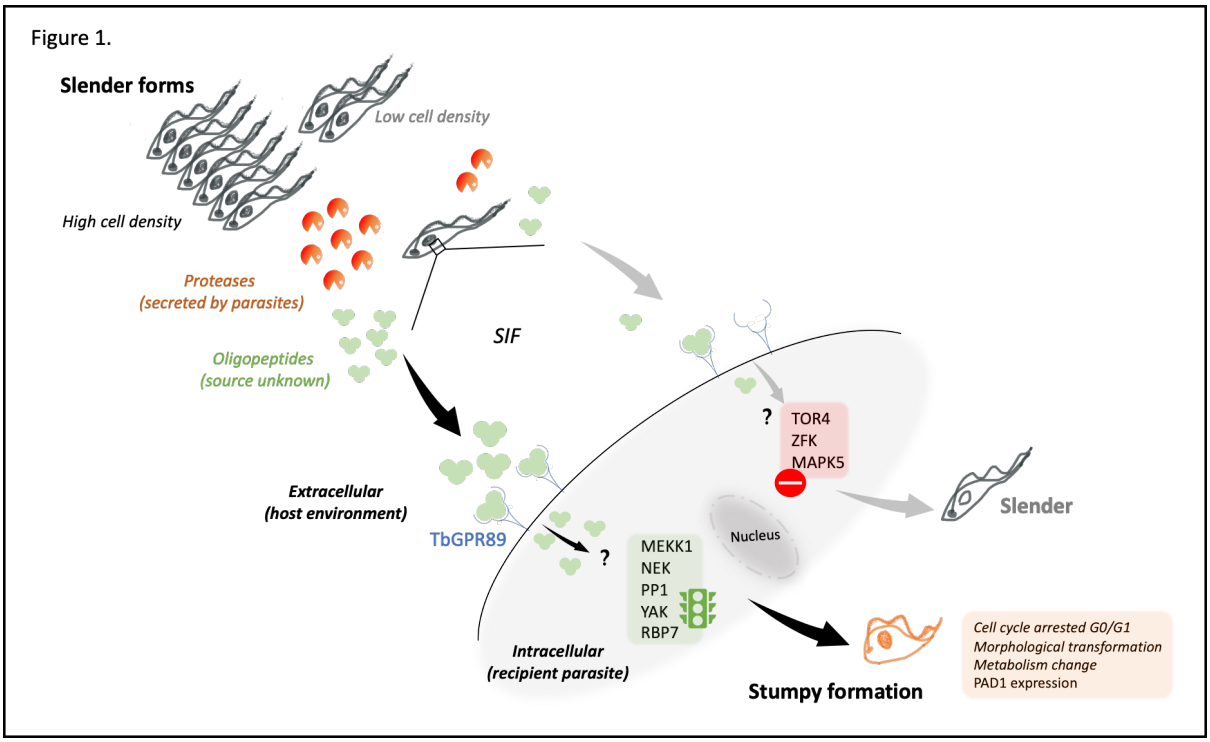

Figure 1. A putative model of stumpy formation based on the recent findings. Parasite density correlates with secreted protease activity, and hence the levels of oligopeptides in the extracellular environment of the parasite. These oligopeptides, the putative SIF, are sensed and internalized by recipient parasites through the putative SIF receptor, TbGPR89. At high parasitemia accumulation of oligopeptides activates signaling pathways (e.g., MEEK1, NEK1, PP1, YAK, RBP7…) that lead to stumpy formation. At lower cell density, less oligopeptides are produced and internalized thus activating an alternative pathway (e.g., TOR4, ZFK, MAPK5 …) that represses stumpy formation and keeps the cell in a replicative state, the slender form.

References

[1] B. Reuner, E. Vassella, B. Yutzy, and M. Boshart, “Cell density triggers slender to stumpy differentiation of Trypanosoma brucei bloodstream forms in culture,” *Mol. Biochem. Parasitol.*, vol. 90, no. 1, pp. 269–280, 1997.

[2] E. Vassella, B. Reuner, B. Yutzy, and M. Boshart, “Differentiation of African trypanosomes is controlled by a density sensing mechanism which signals cell cycle arrest via the cAMP pathway,” *J. Cell Sci.*, vol. 110, no. 21, p. 2661 LP-2671, Nov. 1997.

[3] B. M. Mony *et al.*, “Genome-wide dissection of the quorum sensing signalling pathway in Trypanosoma brucei,” *Nature*, vol. 505, p. 681, Dec. 2013.

[4] L. McDonald *et al.*, “Non-linear hierarchy of the quorum sensing signalling pathway in bloodstream form African trypanosomes,” *PLOS Pathog.*, vol. 14, no. 6, p. e1007145, Jun. 2018.

[5] D. TETAERT, B. SOUDAN, G. HUET-DUVILLIER, P. DEGAND, and A. BOERSMA, “Unusual cleavage of peptidic hormones generated by trypanosome enzymes released in infested rat serum,” *Int. J. Pept. Protein Res.*, vol. 41, no. 2, pp. 147–152, Nov. 2018.

[6] A. Geiger *et al.*, “Exocytosis and protein secretion in Trypanosoma,” *BMC Microbiol.*, vol. 10, no. 1, p. 20, Jan. 2010.

[7] G. Bossard, G. Cuny, and A. Geiger, “Secreted proteases of Trypanosoma brucei gambiense: Possible targets for sleeping sickness control?,” *BioFactors*, vol. 39, no. 4, pp. 407–414, Mar. 2013.

[8] P. Capewell *et al.*, “The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes,” *Elife*, vol. 5, p. e17716, 2016.

[9] S. Trindade *et al.*, “Trypanosoma brucei Parasites Occupy and Functionally Adapt to the Adipose Tissue in Mice,” *Cell Host Microbe*, vol. 19, no. 6, pp. 837–848, Jun. 2016.

[10] N. M. B. Brancucci *et al.*, “Lysophosphatidylcholine Regulates Sexual Stage Differentiation in the Human Malaria Parasite Plasmodium falciparum,” *Cell*, vol. 171, no. 7, p. 1532–1544.e15, Dec. 2017.