that humans are an extraordinarily efficient amplification vector. We are thus both an amplification *and* a geographic vector, able to support massive parasite numbers and walk them further in a day than an infected mosquito could travel in her lifetime. On the coast in Kenya I met a mother who had bundled her malarious infant into her *kikoi* and carried her 10 km to the clinic for treatment. Effectively, she had vectored parasites into the range of a new population of definitive hosts. Our technologies now make us super-vectors, able to carry parasites from continent to continent in our planes, trains, and automobiles.

Vector biologists, and those doing the crucial work of vector control, are probably groaning inwardly at my terminological pedantry, but good science needs accurate communication. As writers, editors, and presenters we must eschew lazy language. We face a terminological paradox where mosquitoes are the vector for malaria the disease, but – strictly speaking – not the vector for *Plasmodium*, the causative agent of malaria. Perhaps a way through the muddle is to say 'malaria is transmitted by mosquitoes', but avoid saying 'the mosquito is the vector', even if you are a vector biologist!

Plasmodium Is Not an Obligate Intracellular Parasite

Whilst it is true that *Plasmodium* is predominantly intracellular in humans, this is the shorter part of its life cycle and involves only a minority of the myriad morphological forms the parasite can adopt. Most of the life cycle, and most of the remarkable parasite forms, are extracellular. For instance, the above-mentioned male and female gametes, the zygote, the ookinete, the oocyst, the sporoblast mother cell, the midgut sporozoites, and the salivary gland sporozoites (i.e., all the mosquito stages) are extracellular parasites. Even some of the human phases (bloodstream sporozoites and merozoites) are essentially extracellular, albeit ever so briefly. The only intracellular stages in the life cycle are the exoerythrocytic forms within human hepatocytes (namely trophozoites, schizonts, and merozoites – which are sometimes referred to as metacryptozoites but are yet to be shown to be fundamentally different to blood stage merozoites) and the human erythrocytic forms (namely, rings, trophozoites, schizonts, and gametocytes). Extracellular forms thus outnumber intracellular forms by about ten to seven (depending on your definitions of a form). Moreover, the extracellular mosquito sector of the parasite's life cycle (~3 weeks) is 50% longer than the intracellular human sector (~2 weeks). A notable exception is *Plasmodium vivax* hypnozoites, an intracellular liver form that can persist for months or years.

Plasmodium is therefore a facultative intracellular parasite, with a protracted extracellular phase in the definitive (mosquito) host, and a typically shorter intracellular phase in the secondary (human) host, which is also the true vector. Avoid describing *Plasmodium* (or indeed the entire Phylum Apicomplexa) as obligate intracellular parasites, and – these admonitions aside – keep up the good work.

Resources

[i] www.wehi.edu.au/wehi-tv/animation

[1]School of BioSciences, University of Melbourne, VIC 3010, Australia

*Correspondence: gim@unimelb.edu.au (G.I. McFadden).

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References

1. McFadden, G.I. (2012) *Plasmodia* – don't. *Trends Parasitol.* 28, 306
2. Rathnapala, U.L. *et al.* (2017) A novel genetic technique in *Plasmodium berghei* allows liver stage analysis of genes required for mosquito stage development and demonstrates that *de novo* heme synthesis is essential for liver stage development in the malaria parasite. *PLoS Pathog.* 13, e1006396
3. Kantele, A. and Jokiranta, S. (2010) *Plasmodium knowlesi* – the fifth species causing human malaria. *Duodecim* 126, 427–434
4. Kantele, A. and Jokiranta, T.S. (2011) Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clin. Infect. Dis.* 52, 1356–1362
5. Ta, T.H. *et al.* (2014) First case of a naturally acquired human infection with *Plasmodium cynomolgi*. *Malar. J.* 13, 68
6. Lalremruata, A. *et al.* (2015) Natural infection of *Plasmodium brasilianum* in humans: man and monkey share quartan malaria parasites in the Venezuelan Amazon. *EBioMedicine* 2, 1186–1192
7. Jongwutiwes, S. *et al.* (2004) Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. *Emerg. Infect. Dis.* 10, 2211–2213
8. Cox-Singh, J. *et al.* (2008) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin. Infect. Dis.* 46, 165–171
9. Coatney, G.R. (1971) The simian malarias: zoonoses, anthroponoses, or both? *Am. J. Trop. Med. Hyg.* 20, 795–803
10. Dodd, R. (1998) Transmission of parasites by blood transfusion. *Vox Sang.* 74, 161–163
11. Cox, F. (1993) *Modern Parasitology: A Textbook of Parasitology.* (2nd edn), Blackwell Scientific Publications
12. Boyd, M. (1949) *Malariology,* Saunders
13. Scott, R. (1979) *Alien,* 20th Century Fox
14. Dawes, E.J. *et al.* (2009) *Anopheles* mortality is both age- and *Plasmodium*-density dependent: implications for malaria transmission. *Malar. J.* 8, 228
15. Ramasamy, M.S. *et al.* (1997) Interactions of human malaria parasites *Plasmodium vivax* and *P. falciparum*, with the midgut of *Anopheles* mosquitoes. *Med. Vet. Entomol.* 11, 290–296
16. White, N.J. and Pongtavornpinyo, W. (2003) The *de novo* selection of drug-resistant malaria parasites. *Proc. Biol. Sci.* 270, 545–554

Spotlight

A Major Step towards Defining the Elusive Stumpy Inducing Factor in *Trypanosoma brucei*

Lauriane Sollelis[1] and Matthias Marti[1,2,*]

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 (Cell 176, 1–12) 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 nonreplicative 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 nonlinear hierarchy of the SIF pathway [4]. So far, the nature of SIF has remained elusive.

A recent study by Matthews and colleagues represents a major step towards defining the SIF pathway [5]. In their study the *T. brucei* orthologue 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, POTs), 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 orthologue. Trypanosomes are known to secrete peptidases into their environment during infection, generating unusual oligopeptides in the blood stream [6]. Indeed, analysis of the secretome of *T. brucei* identified multiple secreted peptidases [7], with a proposed function in modulating host immunity and pathogenesis [8]. The new study by Matthews and colleagues demonstrates that TbGPR89 is an oligotransporter for oligopeptide substrates that are produced by secreted peptidases [5]. 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 internalized by TbGRP89 and can subsequently lead to stumpy formation, recapitulating all the hallmarks of SIF.

The study by Matthews and colleagues 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 [5]. The authors propose a model where levels of secreted peptidases (essentially a proxy for parasitaemia) 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 they 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 a mouse model where a significantly higher parasitaemia 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 parasites homing to skin [9] and adipose tissues [10] 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) [11]. 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

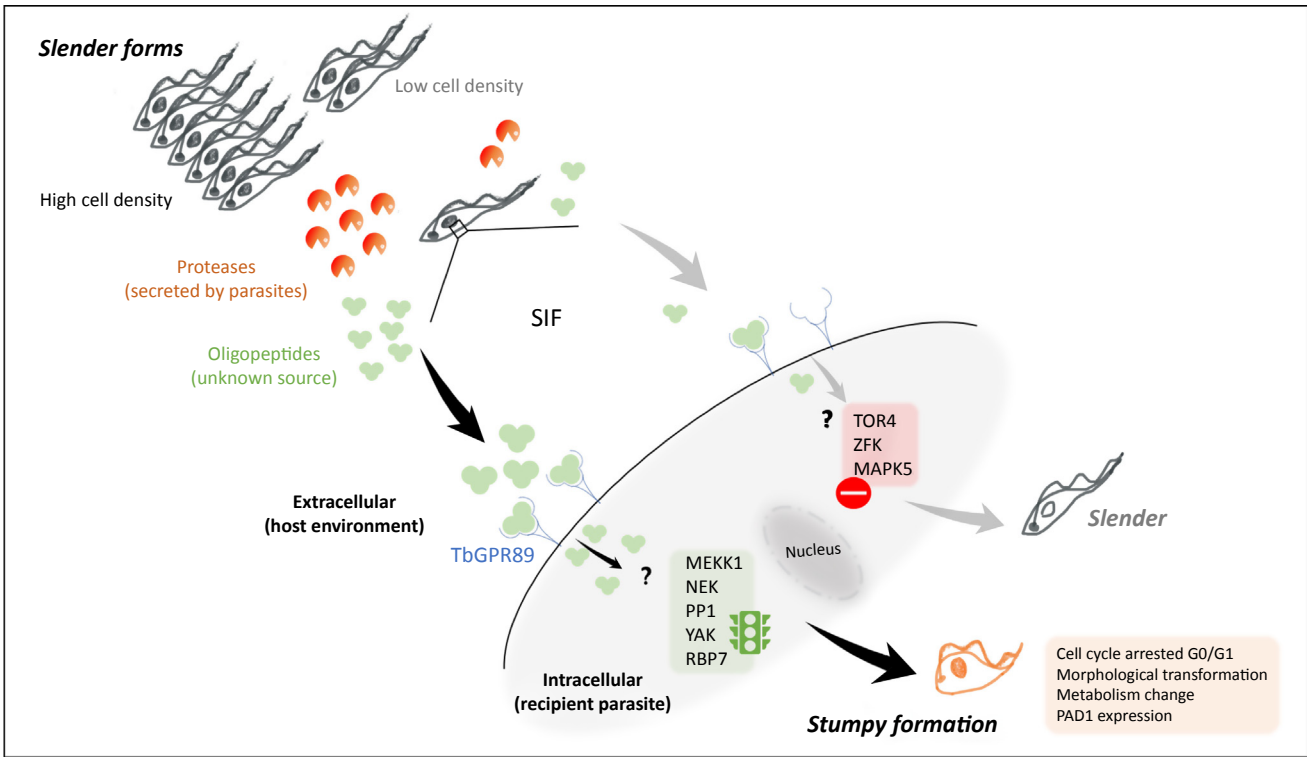

Figure 1. A Putative Model of Stumpy Formation Based on Recent Findings. Parasite density correlates with secreted protease activity, and hence the levels of oligopeptides in the extracellular environment of the parasite. These oligopeptides, the putative stumpy induction factor (SIF), are sensed and internalized by recipient parasites through the putative SIF receptor, TbGPR89. At high parasitaemia, accumulation of oligopeptides activates signalling pathways (e.g., MEEK1, NEK1, PP1, YAK, RBP7 . . .) that lead to stumpy formation. At lower cell density, fewer 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.

development, as TbGRP89 is essential for both parasite survival and stumpy formation and a TbGRP89-targeting drug could potentially reduce parasite burden and at the same time block transmission.

[1]Wellcome Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK
[2]Department of Immunology and Infectious Disease, Harvard T.H. Chan School of Public Health, Boston, MA, USA

*Correspondence:
Matthias.Marti@glasgow.ac.uk (M. Marti).

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References

1. Reuner, B. *et al.* (1997) Cell density triggers slender to stumpy differentiation of *Trypanosoma brucei* bloodstream forms in culture. *Mol. Biochem. Parasitol.* 90, 269–280
2. Vassella, E. *et al.* (1997) Differentiation of African trypanosomes is controlled by a density sensing mechanism which signals cell cycle arrest via the cAMP pathway. *J. Cell Sci.* 110, 2661–2671
3. Mony, B.M. *et al.* (2013) Genome-wide dissection of the quorum sensing signalling pathway in *Trypanosoma brucei*. *Nature* 505, 681
4. McDonald, L. *et al.* (2018) Non-linear hierarchy of the quorum sensing signalling pathway in bloodstream form African trypanosomes. *PLoS Pathog.* 14, e1007145
5. Rojas, F. *et al.* (2019) Oligopeptide signaling through *Tb*GPR89 drives trypanosome quorum sensing. *Cell* 176, 1–12
6. Tetaert, D. *et al.* (2018) Unusual cleavage of peptidic hormones generated by trypanosome enzymes released in infested rat serum. *Int. J. Pept. Protein Res.* 41, 147–152
7. Geiger, A. *et al.* (2010) Exocytosis and protein secretion in *Trypanosoma*. *BMC Microbiol.* 10, 20
8. Bossard, G. *et al.* (2013) Secreted proteases of *Trypanosoma brucei gambiense*: possible targets for sleeping sickness control? *BioFactors* 39, 407–414
9. Capewell, P. *et al.* (2016) The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. *eLife* 5, e17716
10. Trindade, S. *et al.* (2016) *Trypanosoma brucei* parasites occupy and functionally adapt to the adipose tissue in mice. *Cell Host Microbe* 19, 837–848
11. Brancucci, N.M.B. *et al.* (2017) Lysophosphatidylcholine regulates sexual stage differentiation in the human malaria parasite *Plasmodium falciparum*. *Cell* 171, 1532–1544.e15

Forum

Gene Function Discovery for Kinetoplastid Pathogens

Reza Salavati[1,2,3,*] and Vahid H. Gazestani[4]

We propose to integrate the existing and new experimental data