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The Social Life of African Trypanosomes

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Review

The Social Life of African Trypanosomes

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The unicellular parasite *Trypanosoma brucei* shuttles between its definitive host, the tsetse fly, and various mammals including humans. In the fly digestive tract, *T. brucei* must first migrate to the ectoperitrophic space, establish a persistent infection of the midgut and then migrate to the salivary glands before being transmitted to a new mammalian host. In 2010, it was shown that insect stages of the parasite (procyclic forms) exhibit social motility (SoMo) when cultured on a semi-solid surface, and it was postulated that this behaviour might reflect a migration step in the tsetse fly. Now, almost 5 years after the initial report, several new publications shed some light on the biological function of SoMo and provide insights into the underlying signalling pathways.

Trends

Trypanosoma brucei is a unicellular parasite that cycles between tsetse flies and mammals; it is the causative agent of human sleeping sickness and one form of the cattle disease Nagana.

Early procyclic forms are found in the midgut in the first week after trypanosomes are transmitted to tsetse flies; these differentiate to late procyclic forms that persistently infect the midgut.

Early procyclic forms exhibit social behaviour, manifested as coordinated group migration, when cultured on a semi-solid surface. Late procyclic forms do not migrate under these conditions.

cAMP is a key signalling molecule regulating social motility

A mutant with defects in social motility shows impaired colonisation of the fly midgut.

Social Interactions and Cooperative Behaviour

Growing numbers of microorganisms have been shown to socialise with their own species and form multicellular structures. Social behaviour can manifest itself as the aggregation of individuals into communities or as the coordinated group movement of a population. It can also provide signals for a subset of cells within the population to differentiate and assume new functions, such as spore formation or filamentous growth. Cooperation is the quintessence of social behaviour, enhancing the prospects of survival under conditions where single cells might not fare as well. Examples of social interactions include swarming motility, quorum sensing, and biofilm formation. These are well studied in bacteria [1–5] and in the free-living amoeba *Dictyostelium discoideum* [6], but apart from quorum sensing [7–9], are not widely recognised in trypanosomes. Five years ago, the first report that *Trypanosoma brucei* exhibited social motility (SoMo) when plated on a semi-sold surface [10] was met by the research community with enthusiasm and scepticism in approximately equal measures. Did SoMo reflect one or more events during the development of the parasite in its insect host, the tsetse fly? If so, which events? Or might it be a phenomenon that only occurred *in vitro* [11] or, in the worst case, an artefact caused by something as trivial as a film of liquid on the plates? Recent publications strongly support that SoMo is a reflection of a specific phase in the trypanosome life cycle and give the first clues to the pathways involved.

Life Cycle and Cell Architecture of *Trypanosoma brucei*

Trypanosoma brucei subsp are unicellular eukaryotes that cause sleeping sickness in humans or Nagana in domestic animals. The number of cases of human sleeping sickness reported to the World Health Organisation (WHO) currently lies at approximately 7000 per year [12], but it is estimated that the true figure is closer to 20 000. If not treated, the disease is usually fatal. The distribution of *T. brucei* subsp is limited to sub-Saharan Africa, the habitat of the definitive host, the tsetse fly. Both male and female tsetse can acquire trypanosomes when they take a blood meal from an infected mammal. The most challenging part of the life cycle is the development of the parasite in the tsetse fly digestive tract and the production of infectious forms in the salivary glands that can then be transmitted to new mammalian hosts [13] (Figure 1). Even under optimised conditions in the laboratory, when flies are infected during their first blood meal, fewer

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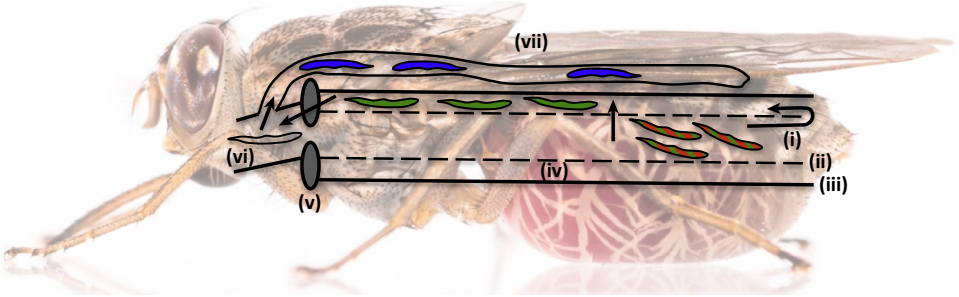

Figure 1. Schematic Diagram of the Migration of *Trypanosoma brucei* Through the Tsetse Fly. The tsetse fly shown has just taken a blood meal. Numbers indicate: (i) midgut lumen; (ii) peritrophic matrix; (iii) midgut epithelium; (iv) ectoperitrophic space; (v) proventriculus; (vi) oesophagus; and (vii) salivary glands. Colours indicate the stage-specific surface glycoproteins GPEET procyclin (red) and EP procyclin (green) expressed by procyclic forms in the midgut and *brucei* alanine-rich protein (BARP; blue) expressed by epimastigote forms in the salivary glands [24]. It is not known whether procyclic-form trypanosomes penetrate the peritrophic matrix or migrate around it.

than 20% will develop a mature infection, allowing them to transmit trypanosomes; in the wild, this number is considerably lower [14]. Following a blood meal, trypanosomes are found in the midgut lumen [15], where they differentiate to early procyclic forms [16]. Many flies are able to clear the infection at this point [15]. To survive and colonise the midgut, the trypanosomes must migrate to the other side of the peritrophic matrix (PM), a chitinous structure that serves as a physical barrier between the blood meal and the insect gut epithelium. The pores in the PM, which are approximately 9 nm in diameter [17], are too narrow for the trypanosomes to squeeze through, implying that they either have to breach the PM or circumvent it to reach the ectoperitrophic space. Six days post infection, most trypanosomes are seen in the ectoperitrophic space of infected flies and few are in the lumen [15]. By this time, most of these parasites are late procyclic forms [16]. Studies with tagged trypanosomes indicate that the ectoperitrophic space can be colonised by a founding population of several hundred trypanosomes [18], but it is not known whether these migrate individually or in groups. Even when colonisation of the midgut is successful, many infections fail to progress beyond this stage [19]. To complete the life cycle, parasites must move forward to the anterior midgut, gain access to the lumen of the proventriculus, and then invade the salivary glands. This migration represents a major bottleneck, with only a few founder cells colonising the glands [18]. The transmission cycle is completed in the salivary glands with the formation of infectious metacyclic forms that are transferred to a new mammalian host.

Two hallmarks of trypanosomes are their highly polarised cell structure [20,21] and their stage-specific coat proteins [16,22–24]. Each cell has a single flagellum that is attached along the length of the cell body. The tip of the flagellum, which extends beyond the cell body, is the anterior pole of the cell; flagellar beating pulls the cell body forward, the tip leading, and determines the direction of movement [25]. The cell surface of different life-cycle stages is dominated by specific glycosylphosphatidylinositol (GPI)-anchored proteins (Figure 1). Bloodstream forms in the mammalian host are covered by a coat of variant surface glycoprotein (VSG) that is shed when the trypanosomes differentiate to procyclic forms [26]. In the fly, VSG is replaced by EP and GPEET procyclins [27], which are named after their internal dipeptide (EP) and pentapeptide (GPEET) repeats. Early procyclic forms, which are detected in the fly midgut during the first week of infection, express high levels of GPEET and lower levels of EP, whereas late procyclic forms, which persistently colonise the ectoperitrophic space, express higher levels of EP than early forms, but are negative for GPEET [16,27]. The progression from early to late procyclic forms is irreversible in the fly [16,27]. In culture, however, differentiation can occur in

both directions [28,29] and only some of the factors that cause this are known. Early and late procyclic culture forms are morphologically indistinguishable and, until recently [30], GPEET was the only marker that could discriminate between them.

The Discovery of SoMo

Figure 2 shows an example of SoMo when wild-type procyclic forms are spotted onto a plate and photographed at daily intervals. That SoMo was discovered at all was due to a fortuitous combination of the right environment and sharp powers of observation. Procyclic-form trypanosomes are normally grown in suspension cultures. Kent Hill's laboratory was studying trypanosome motility and was working on ways to develop new assays for this. Based on the knowledge that bacterial motility mutants show altered colony morphologies and sizes on agarose plates, together with the idea that trypanosomes in the tsetse are in close contact with surfaces, the authors adapted an agarose-plating procedure used to clone trypanosomes [31]. In the process, they observed that cells initially formed colonies of varying size and that these could move across the surface. Our laboratory (and possibly many others) had tried to clone procyclic forms by plating, but abandoned it because the cells formed streaks rather than individual colonies. At the time, we thought that this was caused by moisture on the plates. What the Hill laboratory suspected, however, was that colony movements could be an indicator of social behaviour and, rather than consigning their plates to the autoclave, as we did, they investigated the phenomenon more closely. By using time-lapsed and live video microscopy, it was shown that trypanosome colonies did not form simply through clonal expansion. Rather, individual cells first collected into colonies at the inoculation site and cells within a colony moved as a group across the surface and were able to collect individuals from their surroundings [10]. Colonies could also merge with each other and this, together with continued cell division, led to

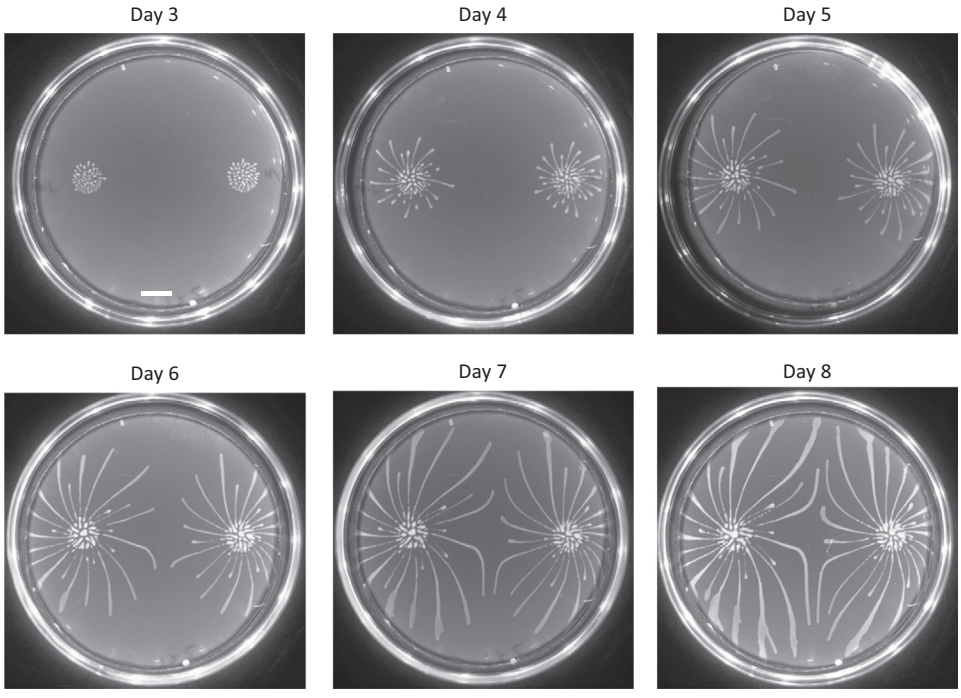

Figure 2. Social Media: The Behaviour of *Trypanosoma brucei* on a Semi-Solid Surface. Procyclic-form trypanosomes (two aliquots from the same liquid culture) were inoculated onto a plate containing 0.4% agarose. Migration started at day 3 post inoculation; the plate was photographed every 24 h from days 3 to 8. From day 6 onwards, it can be seen that radial projections from the two communities change direction to avoid touching each other. Scale bar = 1 cm.

large colonies visible to the naked eye. Following suggestions of a colleague studying social behaviour in bacteria, Hill's group allowed surface-cultivated trypanosomes to continue growing for several generations and monitored the perimeter of the colony, because that is where bacterial mutants often are distinguished from wild-type cells. At the perimeter of the colony, cells formed streams (also known as radial projections, protrusions, or fingers), which moved en masse away from the inoculation site [10]. Cells within these streams moved freely in all directions, but there was a net outward movement of about 1 cm per day, corresponding to approximately 500 body lengths [10]. The behaviour was termed SoMo based on similarities to swarming motility in bacteria.

Single cells are unable to move efficiently over the agarose surface. Moreover, a functional flagellum is essential for SoMo, indicating that migration is an active process [10]. Projections form when the cells reach a threshold number of approximately 1.6 million [30]. The spacing between projections is usually constant for a given community, suggesting that there are factors determining the position where migration starts. The projections are initially straight, but when they are allowed to grow unimpeded, they gradually spiral clockwise [10,30]. When two streams come into close proximity, they change direction to avoid contact, indicating that they are reacting to a repellent [10]. Based on this behaviour, it would seem that several signals are involved: one that attracts the parasites to each other, one that holds them together within a stream, and one that drives the streams away from the inoculation site. In theory, the latter could also be the repellent that causes migrating streams to avoid other trypanosome communities. The signal that coordinates group movement could be a short-range diffusible factor or direct cell–cell contact. It is possible that the signal that drives them outwards is a diffusible secreted factor that can operate over relatively long distances, and that the same signal determines the distance between adjacent streams.

SoMo Is Restricted to Early Procyclic Forms and Correlates with Establishment of Midgut Infections

Since trypanosomes migrate on two occasions in the tsetse fly, once to colonise the midgut and then to invade the salivary glands, it is an attractive proposition that SoMo reflects one of these events. Several lines of evidence support the notion that SoMo corresponds to the former. First, only early procyclic forms are capable of migrating on plates [30]. Staining of communities with anti-GPEET and anti-EP antibodies showed that the protrusions are positive for GPEET, while the cells remaining at the inoculation site are GPEET-negative and, thus, are late procyclic forms (Figure 3). Neither GPEET nor EP is required for the three key features of SoMo (migration, avoidance, and repellent production [30]). Rather, it is the developmental status of the cells (late versus early procyclic forms) that is reflected in the failure of GPEET-negative cells to engage in SoMo. Comparison of the proteomes of these two life-cycle stages has led to the identification of several other proteins that are differentially expressed, including calflagins, nucleoside and amino acid transporters, metabolic enzymes, and the inevitable hypothetical proteins [30]. Whether any of these have a role in SoMo remains to be established.

The first evidence for a connection between SoMo and successful colonisation of the midgut came from a mutant lacking the *Rft1* gene [32]. Rft1 has a role in *N*-linked glycosylation and a null mutant showed reduced binding by the lectin Concanavalin A, indicating a paucity of mannose residues [33]. This mutant also showed two phenotypes in SoMo: it formed fewer radial projections than its wild-type parent and needed to reach a higher threshold cell number before migration was initiated [32]. Both of these point to a defect in either the production or the perception of a migration-stimulating factor. The recognition and avoidance of oncoming projections appeared normal, however, indicating that the mutant can still synthesise and react to the repellent [32]. This is the only evidence, so far, that the repellent and migration factors might be different entities. In tsetse, the *Rft1* null mutant was able to survive as well as the wild

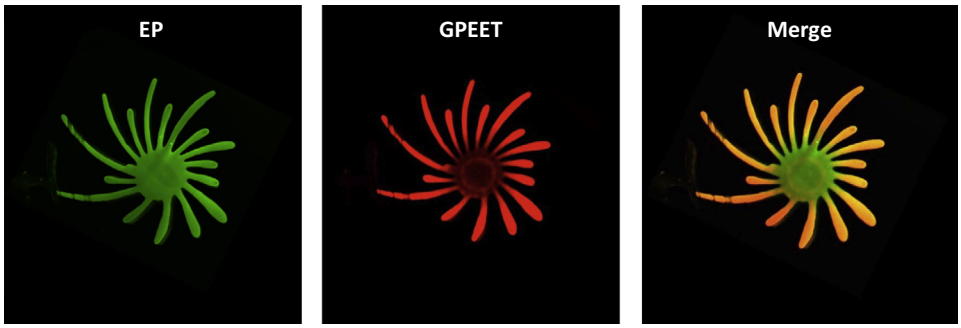

Figure 3. GPEET Procyclin, a Marker for Early Procyclic Forms, Discriminates Between Migrating and Sedentary Trypanosomes. The developmental status of cells on a plate can be determined by performing a 'community lift' [30,50]. This entails placing a nitrocellulose filter onto the plate once the cells have grown. The cells adhere to the filter and can be labelled with anti-EP and anti-GPEET antibodies. GPEET-negative cells (late procyclic forms) accumulate in the middle of the community, while GPEET-positive cells (early procyclic forms) migrate away from the middle.

type 3 days post infection, but it showed a lower frequency of established midgut infections 14 days post infection [32]. When infections did occur, they were heavy, implying that once the parasites gain access to the ectoperitrophic space, they can grow normally. In the fly, late procyclic forms differentiate to mesocyclic forms, which are the forerunners of long epimastigote forms that migrate to the salivary glands. Since *Rft1* was knocked out in a stock of *T. brucei* that does not give rise to salivary gland infections [34], it is not known how a null mutant would behave during migration from the midgut to the salivary glands. There is evidence, however, that SoMo can be uncoupled from colonisation of the glands: a knockout of mitogen-activated protein (MAP) kinase kinase 1 in a fly transmissible strain completely abolished its ability to infect the salivary glands, while having no effect on midgut infections or SoMo [30,35]. Similarly, a knockout of the transmembrane protein procyclic-specific surface antigen 2 (PSSA-2) showed impaired colonisation of the glands, but normal SoMo and midgut infections [30,36].

SoMo Is Regulated by Adenylyl Cyclases and a Phosphodiesterase

Cyclic mono- and dinucleotides are frequently used by microbes as second messengers. Cyclic di-GMP is used by many bacteria to regulate biofilm formation and swarming [37–39]. *Dictyostelium discoideum* emits waves of cAMP that promote aggregation and slug formation (reviewed in [6]), while cyclic di-GMP is required for differentiation into stalk cells and spores [40]. Many signals in eukaryotic cells are transmitted by G protein-coupled receptors, but these are not found in trypanosomes. Instead, *T. brucei* encodes an unusually large number of receptor adenylyl cyclases (AC), indicating that cAMP is likely to be a second messenger in many signalling pathways. Trypanosomal ACs have a large extracellular N-terminal domain, a single membrane-spanning region, and a cytoplasmic catalytic domain. In this respect, they show more similarity to bacterial guanylyl cyclases than to mammalian AC, which are multi-pass membrane-spanning proteins without intrinsic receptor capacity. It has been shown, however, that several of these have the amino acid signatures of AC [41,42], catalyse formation of cAMP, and rescue AC mutants in yeast [43,44]. To date, no ligands for these receptors have been identified.

The prototypical ACs in *T. brucei*, which constitute the ESAG4 family, are part of the VSG expression sites that are expressed only in bloodstream forms. In addition, there are 65 genes related to ESAG4 (GRESAG4s) that are distributed over the 11 pairs of megabase chromosomes. Until recently, it was assumed that the many versions of GRESAG4 were constitutively expressed, but there are now several examples of stage-regulated AC. Through a combination of proteomics and northern blot analysis, it was shown that closely related ACs are differentially expressed between early and late procyclic forms [30]. In addition, proteomics and quantitative

reverse transcription PCR (qRT-PCR) identified six AC, designated ACP1–6, that were detected in procyclic forms but not bloodstream forms [44]. In the latter study, it is not known whether the procyclic forms were early, late, or a mixture of the two.

Intriguingly, knockdown of ACP6 by RNA interference resulted in a hypersocial phenotype with the production of more radial projections compared with the parental stock, suggesting that decreased levels of cAMP favour SoMo [45]. The same phenotype was observed after dual knockdown of ACP1 and 2 [45]; these two ACs are closely related, but diverge at their C termini and are differentially localised to the tip and entire flagellum, respectively [44]. At first sight, it might seem puzzling that the stock used by Lopez *et al.* [45] produced fewer than half the number of radial projections seen by Imhof *et al.* [32], although both were derived from *T. b. brucei* Lister 427; in fact, based on the number of projections, the hypersocial mutants more closely resembled the wild type used by Imhof and coworkers. One explanation for this might be that the two populations contained different proportions of early and late procyclic forms. The stock used by Imhof *et al.* consisted predominantly of early procyclic forms (>90% GPEET-positive), while Lopez and coworkers did not discriminate between early and late procyclics. Comparative proteomics of early and late procyclic forms indicated that either ACP1 and/or ACP2 were fivefold more abundant in late procyclic forms [30]. Since the 3′ untranslated regions (UTRs) of the two transcripts are unique, however, this allows them to be distinguished by qRT-PCR [45] and RNA-Seq. ACP1 mRNA is five times more abundant in late procyclic forms, whereas ACP2 is reduced almost 2-fold. ACP6 was not detected by stable isotope labelling by

Key Figure

Schematic Depiction of Phosphodiesterase PDEB1 and Adenylyl Cyclases ACP1, 2, and 6 on the Flagellum of *Trypanosoma brucei* and the Effect of RNA interference on Social Motility (SoMo)

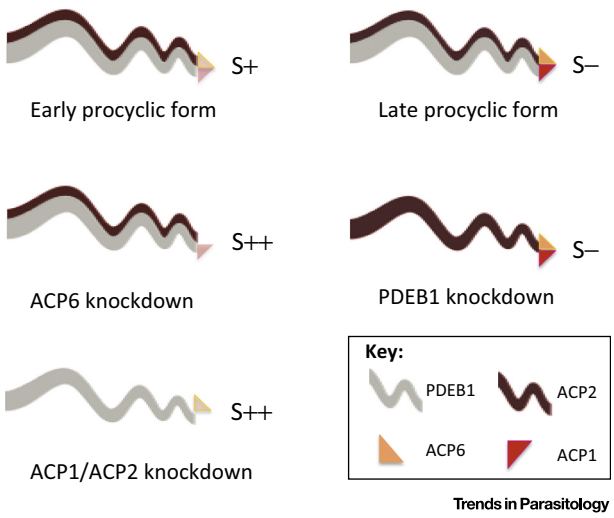

Figure 4. Phenotypes are indicated as: S–, SoMo negative; S+, SoMo positive; S++, hypersocial. Based on RNA-Seq, ACP1 (Tb927.11.17040) and ACP6 (Tb927.9.15660) are upregulated fivefold and 2.5-fold, respectively, in late procyclic forms; ACP2 (Tb927.10.16190) is downregulated approximately 2-fold[i]. The scheme assumes that there is some basal activity of ACP1 and ACP6 in early procyclic forms. Knockdown of PDEB1 (Tb927.9.5040) leads to an increased concentration of cAMP at the flagellar tip, inhibiting SoMo. Conversely, knockdown of either ACP1 and ACP2, or ACP6 leads to a reduction of cAMP at the tip, enabling parasites to become hypersocial.

amino acids in cell culture (SILAC) [30], but the transcript was 2.5-fold more abundant in late procyclic forms[i]. Therefore, consistent with the model proposed by Lopez *et al*., we speculate that upregulation of ACP1 and ACP6 in late procyclic forms, and the resulting local increase in cAMP at the tip of flagellum, act as a brake for SoMo. Knocking down either of these ACs might cause late procyclic forms within a population to revert to a more 'early' state that is able to perform SoMo or render early procyclic forms hypersocial (Figure 4, Key Figure). Whether ACP1 and ACP6 act independently or in concert remains to be established. Trypanosomal ACs have the capacity to dimerise [43,44], but it is not yet known whether ACP1 and ACP6 are part of a single complex that operates as a functional unit. In this context, other ACs that are stage-regulated early and late procyclic forms also merit further investigation. Peptides derived from either AC330 (which is upregulated in early procyclic forms) or AC320/AC285 (which are upregulated in late procyclic forms) [30] have been detected in the flagellar proteome [46]. These should be localised and tested for a role in SoMo.

Since cAMP is a small molecule with a high diffusion coefficient, and trypanosomes have a length of 20 μm and a diameter of only 2 μm, cAMP would rapidly equilibrate throughout the cell unless there was a stringent system of control [47]. The fact that all ACs that have been localised to date are in the flagellum [44], together with the finding that the cAMP-specific phosphodiesterase PDEB1 is distributed along its entire length [48], led to the concept of signalling microdomains with the phosphodiesterase acting as a diffusion barrier [49]. In keeping with this model, knocking down PDEB1 or plating cells in the presence of a phosphodiesterase inhibitor increased intracellular levels of cAMP and rendered the communities completely negative for SoMo [50]. The parasites were still GPEET-positive, however, and exhibited normal motility. Interestingly, the defect in SoMo could be complemented by plating the PDEB1 mutant together with wild-type cells [50]. The simplest interpretation of these results is that the mutant is still receptive to migration factors, but does not produce them.

Open Questions

In their original publication about SoMo, Oberholzer *et al.* postulated that it might be manifestation of an event in the trypanosome life cycle [10]. The recent finding that SoMo is restricted to early procyclic forms [30], which are found in the tsetse fly for the first few days after they become infected, lends credence to this proposition. It is encouraging that the *Rft1* knockout exhibits defects in both SoMo and colonisation of the tsetse midgut, indicating that the two phenomena are linked [32], but more mutants need to be tested *in vivo* to consolidate or refute the hypothesis. Early events following fly infection should also be investigated in more detail. For example, it is not known whether trypanosomes move to the ectoperitrophic space individually or as cohorts. Determining the identity of the migration stimulating factor(s) should be high on the to-do list. A comprehensive analysis of the metabolomes and the secretomes of early and late procyclic forms would be a first step towards pinpointing candidates. In this regard, the PDEB1-knockdown mutant, which appears to be deficient in the production of such factors, is a valuable resource. The nature and the function of the repellents produced by communities are also unknown and are equally deserving of closer scrutiny. It has already been shown that surface proteins, the ACs, can regulate the response to SoMo and that the major surface proteins are dispensable. A thorough comparison of the surface proteomes of early and late procyclic forms, with attention to differences in post-translational modifications, is also likely to provide further insights into the response mechanisms (see Outstanding Questions Box).

Concluding Remarks

After a hiatus of several years following the first report on SoMo [10], four new publications have appeared within a few months of each other [30,32,45,50]. These not only pin down several genes involved in social behaviour, but also identify the mode of signal transduction [45,50]. An important concept to emerge from these studies is that parasite–parasite interactions are likely

Outstanding Questions

Is SoMo a manifestation of an event in the trypanosome life cycle?

Are SoMo and colonisation of the tsetse midgut linked?

Do trypanosomes move to the ectoperitrophic space individually or as cohorts?

What is the identity of the migration and repellent factors?

How is SoMo regulated?

to be every bit as important as host–parasite interactions for successful transmission by tsetse [32]. It is also important to recognise that culture conditions can have a major impact on behaviour. A number of the discoveries reported here were made with pre-existing mutants that showed modest or no phenotypes in liquid culture. Incorporating SoMo into the (currently limited) palette of assays used to characterise mutants might prove rewarding, with the proviso that cells should always first be typed as early or late procyclic forms to avoid unnecessary pitfalls.

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Resources

[i] http://tritrypdb.org/tritrypdb/

References

1. Bassler, B.L. and Losick, R. (2006) Bacterially speaking. *Cell* 125, 237–246
2. Vlamakis, H. *et al.* (2013) Sticking together: building a biofilm the *Bacillus subtilis* way. *Nat. Rev. Microbiol.* 11, 157–168
3. Laverty, G. *et al.* (2014) Biomolecular mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* biofilm formation. *Pathogens* 3, 596–632
4. Berleman, J.E. and Kirby, J.R. (2009) Deciphering the hunting strategy of a bacterial wolfpack. *FEMS Microbiol. Rev.* 33, 942–957
5. Kaiser, D. (2003) Coupling cell movement to multicellular development in myxobacteria. *Nat. Rev. Microbiol.* 1, 45–54
6. Loomis, W.F. (2014) Cell signaling during development of *Dictyostelium*. *Dev. Biol.* 391, 1–16
7. 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
8. Mony, B.M. *et al.* (2014) Genome-wide dissection of the quorum sensing signalling pathway in *Trypanosoma brucei*. *Nature* 505, 681–685
9. Mony, B.M. and Matthews, K.R. (2015) Assembling the components of the quorum sensing pathway in African trypanosomes. *Mol. Microbiol.* 96, 220–232
10. Oberholzer, M. *et al.* (2010) Social motility in African trypanosomes. *PLoS Pathog.* 6, e1000739
11. Bastin, P. and Rotureau, B. (2015) Social motility in African trypanosomes: fact or model? *Trends Parasitol.* 31, 37–38
12. WHO (2014) *Fact Sheet No. 259: Trypanosomiasis, Human African (Sleeping Sickness)*, WHO
13. Roditi, I. and Lehane, M.J. (2008) Interactions between trypanosomes and tsetse flies. *Curr. Opin. Microbiol.* 11, 345–351
14. Haines, L.R. (2013) Examining the tsetse teneral phenomenon and permissiveness to trypanosome infection. *Front. Cell. Infect. Microbiol.* 3, 1–6
15. Gibson, W. and Bailey, M. (2003) The development of *Trypanosoma brucei* within the tsetse fly midgut observed using green fluorescent trypanosomes. *Kinetoplastid Biol. Dis.* 2, 1
16. Vassella, E. *et al.* (2000) A major surface glycoprotein of *Trypanosoma brucei* is expressed transiently during development and can be regulated post-transcriptionally by glycerol or hypoxia. *Genes Dev.* 14, 615–626
17. Miller, N. and Lehane, M.J. (1993) Peritrophic membranes, cell surface molecules and parasite tropisms within arthropod vectors. *Parasitol. Today* 9, 45–50
18. Oberle, M. *et al.* (2010) Bottlenecks and the maintenance of minor genotypes during the life cycle of *Trypanosoma brucei*. *PLoS Pathog.* 6, e1001023
19. Van Den Abbeele, J. *et al.* (1999) *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. *Parasitology* 118, 469–478
20. Hemphill, A. *et al.* (1991) The cytoskeletal architecture of *Trypanosoma brucei*. *J. Parasitol.* 77, 603–612
21. Gull, K. (1999) The cytoskeleton of trypanosomatid parasites. *Annu. Rev. Microbiol.* 53, 629–655
22. Vickerman, K. and Luckins, A.G. (1969) Localization of variable antigens in the surface coat of *Trypanosoma brucei* using ferritin conjugated antibody. *Nature* 224, 1125–1126
23. Richardson, J.P. *et al.* (1988) Procyclin: an unusual immunodominant glycoprotein surface antigen from the procyclic stage of African trypanosomes. *Mol. Biochem. Parasitol.* 31, 203–216
24. Urwyler, S. *et al.* (2007) A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Mol. Microbiol.* 63, 218–228
25. Rodriguez, J.A. *et al.* (2009) Propulsion of African trypanosomes is driven by bihelical waves with alternating chirality separated by kinks. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19322–19327
26. Roditi, I. *et al.* (1989) Procyclin gene expression and loss of the variant surface glycoprotein during differentiation of *Trypanosoma brucei*. *J. Cell Biol.* 108, 737–746
27. Acosta-Serrano, A. *et al.* (2001) The surface coat of procyclic *Trypanosoma brucei:* programmed expression and proteolytic cleavage of procyclin in the tsetse fly. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1513–1518
28. Morris, J.C. *et al.* (2002) Glycolysis modulates trypanosome glycoprotein expression as revealed by an RNAi library. *EMBO J.* 21, 4429–4438
29. Vassella, E. *et al.* (2004) Expression of a major surface protein of *Trypanosoma brucei* insect forms is controlled by the activity of mitochondrial enzymes. *Mol. Biol. Cell* 15, 3986–3993
30. Imhof, S. *et al.* (2014) Social motility of African trypanosomes is a property of a distinct life-cycle stage that occurs early in tsetse fly transmission. *PLoS Pathog.* 10, e1004493
31. Carruthers, V.B. and Cross, G.A. (1992) High-efficiency clonal growth of bloodstream- and insect-form *Trypanosoma brucei* on agarose plates. *Proc. Natl. Acad. Sci. U.S.A.* 89, 8818–8821
32. Imhof, S. *et al.* (2015) A glycosylation mutant of *Trypanosoma brucei* links social motility defects in vitro to impaired colonisation of tsetse in vivo. *Eukaryot. Cell* 14, 588–592
33. Jelk, J. *et al.* (2013) Glycoprotein biosynthesis in a eukaryote lacking the membrane protein Rft1. *J. Biol. Chem.* 288, 20616–20623

34. Ruepp, S. *et al.* (1997) Survival of *Trypanosoma brucei* in the tsetse fly is enhanced by the expression of specific forms of procyclin. *J. Cell Biol.* 137, 1369–1379
35. Morand, S. *et al.* (2012) MAP kinase kinase 1 (MKK1) is essential for transmission of *Trypanosoma brucei* by *Glossina morsitans*. *Mol. Biochem. Parasitol.* 186, 73–76
36. Fragoso, C.M. *et al.* (2009) PSSA-2, a membrane-spanning phosphoprotein of *Trypanosoma brucei*, is required for efficient maturation of infection. *PLoS ONE* 4, e7074
37. Kirillina, O. *et al.* (2004) HmsP, a putative phosphodiesterase, and HmsT, a putative diguanylate cyclase, control Hms-dependent biofilm formation in *Yersinia pestis*. *Mol. Microbiol.* 54, 75–88
38. Hengge, R. (2009) Principles of c-di-GMP signalling in bacteria. *Nat. Rev. Microbiol.* 7, 263–273
39. Kuchma, S.L. *et al.* (2015) Cyclic di-GMP-mediated repression of swarming motility by *Pseudomonas aeruginosa* PA14 requires the MotAB stator. *J. Bacteriol.* 197, 420–430
40. Chen, Z.H. and Schaap, P. (2012) The prokaryote messenger c-di-GMP triggers stalk cell differentiation in *Dictyostelium*. *Nature* 488, 680–683
41. Tucker, C.L. *et al.* (1998) Two amino acid substitutions convert a guanylyl cyclase, RetGC-1, into an adenylyl cyclase. *Proc. Natl. Acad. Sci. U.S.A.* 95, 5993–5997
42. Sunahara, R.K. *et al.* (1998) Exchange of substrate and inhibitor specificities between adenylyl and guanylyl cyclases. *J. Biol. Chem.* 273, 16332–16338
43. Naula, C. *et al.* (2001) Spontaneous dimerization and leucine-zipper induced activation of the recombinant catalytic domain of a new adenylyl cyclase of *Trypanosoma brucei*, GRESAG4.4B. *Mol. Biochem. Parasitol.* 112, 19–28
44. Saada, E.A. *et al.* (2014) Insect stage-specific receptor adenylate cyclases are localized to distinct subdomains of the *Trypanosoma brucei* flagellar membrane. *Eukaryot. Cell* 13, 1064–1076
45. Lopez, M.A. *et al.* (2015) Insect stage-specific adenylate cyclases regulate social motility in African trypanosomes. *Eukaryot. Cell* 14, 104–112
46. Subota, I. *et al.* (2014) Proteomic analysis of intact flagella of procyclic *Trypanosoma brucei* cells identifies novel flagellar proteins with unique sub-localization and dynamics. *Mol. Cell. Proteomics* 13, 1769–1786
47. Seebeck, T. *et al.* (2001) cAMP signalling in *Trypanosoma brucei*. *Int. J. Parasitol.* 31, 491–498
48. Oberholzer, M. *et al.* (2007) The *Trypanosoma brucei* cAMP phosphodiesterases TbrPDEB1 and TbrPDEB2: flagellar enzymes that are essential for parasite virulence. *FASEB J.* 21, 720–731
49. Oberholzer, M. *et al.* (2007) Trypanosomes and mammalian sperm: one of a kind? *Trends Parasitol.* 23, 71–77
50. Oberholzer, M. *et al.* (2015) cAMP regulates social behavior in African trypanosomes. *MBio* 6, e01954–e2014