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Review

Motility, Force Generation, and Energy Consumption of Unicellular Parasites

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Motility is a key factor for pathogenicity of unicellular parasites, enabling them to infiltrate and evade host cells, and perform several of their life-cycle events. State-of-the-art methods of motility analysis rely on a combination of optical tweezers with high-resolution microscopy and microfluidics. With this technology, propulsion forces, energies, and power generation can be determined so as to shed light on the motion mechanisms, chemotactic behavior, and specific survival strategies of unicellular parasites. With these new tools in hand, we can elucidate the mechanisms of motility and force generation of unicellular parasites, and identify ways to manipulate and eventually inhibit them.

The Importance of Being Motile

Motility is a key factor for pathogenicity of unicellular parasites, enabling intercellular transport and invasion of host cells and tissues relevant to their life cycle (Figures 1,2). Motility of unicellular pathogens depends greatly on their propulsion mechanism, their sizes and shapes, as well as on their local environments during invasion and evasion of host immune systems.

Unicellular parasites propel themselves by various motility strategies and mechanisms through the body fluids and tissues of their host. For microscopic unicellular parasites swimming in a surrounding liquid, inertial forces are negligible, whereas viscous forces are greatly dominating. One of the consequences of the dominance of viscous forces is that cells stop immediately when they are not propelled [1–3]. This lack of inertial forces is often compared to a human trying to swim in honey, tar, or molasses [1]. In viscous force-dominated environments, only **non-reciprocal movements** (see Glossary) lead to a net displacement, whereas a swimmer using reciprocal movements would end at his/her starting position after his/her stroke [1]. Unicellular parasites developed their own techniques for moving in viscous environments; these either include shape distortions, rotating or beating flagella, or adhering to surfaces with retractable organelles such as pili [1,4–8].

The importance of motility has been extensively studied in *Trypanosoma brucei* spp. Motility (and thus a healthy flagellum) is essential for viability [9], morphogenesis [10], cell division [11], and propulsion [12], as well as for immune evasion [13] by these organisms. During their life cycle, trypanosomes change structure and thus their mechanism of motility, and this is necessary to differentiate inside the tsetse fly midgut [14]. Viable trypanosome mutants that could only swim slowly backwards failed to infect the midgut of tsetse flies or differentiate into the epimastigote form [14].

In addition, more complex motility patterns, as of the multi-flagellated parasite *Giardia lamblia*, were investigated and modeled [15]. Flagellar motility is a key factor in *Giardia* pathogenesis and

Trends

The quantification of the propulsion forces and power of unicellular parasites elucidate their motility, prowess to infiltrate cells, and how they cause diseases and antagonize attacks by the immune system.

To quantify forces, energy consumption and power generation, optical tweezers have proven to be a crucial tool. Optical tweezers can hold, displace, and manipulate parasites without actually touching them.

Various strategies to study parasite motility and forces rely on placing parasites in predefined environments, specific confinements, pattern arrays, or chemical gradients.

Motility analysis allows fast and detailed analysis of parasite adaptation to their environment and of genetic aspects of parasite locomotion.

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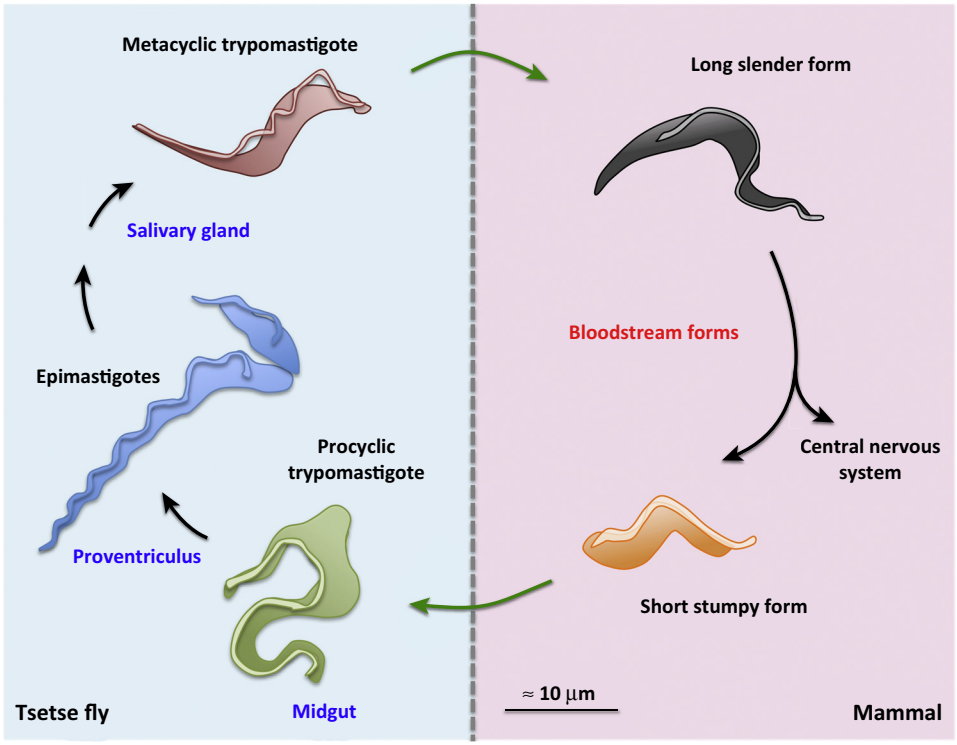

Figure 1. The Life Cycle of *Trypanosoma brucei* [35]. Infection of a mammalian host initiates when a tsetse fly bite delivers growth-arrested metacyclic trypomastigotes to the mammalian bloodstream. Upon entry into the mammalian host, the parasite differentiates into a long, slender, proliferative bloodstream form and multiplies, spreading throughout the body via the circulatory and lymphatic systems. The parasites eventually penetrate blood vessel endothelia and invade the central nervous system. In the bloodstream, some parasites transform into a short stumpy, non-proliferative form. Parasites can in turn be taken up by a tsetse fly when an infected host is bitten. In the tsetse fly, the short stumpy form differentiates into procyclic trypomastigotes, which resume cell division. The procyclic trypomastigote makes an arduous journey via the midgut toward the salivary glands of its vector host, during which the parasites differentiate first into the epimastigote form and finally into non-proliferative metacyclic trypomastigotes. The next blood meal closes the infectious life cycle of *Trypanosoma brucei*.

colonization of the host small intestine [16]. Whereas some of the flagella propel *Giardia lamblia*, others steer its course, with propulsion forces in the lower pN range [17]. Based on a **high-speed imaging** approach, it was shown that *Tritrichomonas foetus* employs similar swimming techniques and forces [18].

In contrast to flagellated unicellular parasites, most apicomplexans, including *Plasmodium* spp. and *Toxoplasma gondii*, exhibit gliding motility as extracellular zoites [19] by using the glideosome as motility machinery [20]. These parasites use the apicomplexan glideosome for intercellular motility as well as invasion of their host cells [20,21]. The invasion is initiated by forming the moving junction [21]. *Plasmodium* motility, essential for tissue penetration and host cell invasion, depends on a sequence of adhesions and releases mediated by the cell apical and proximal ends and by the cell center [22].

At the subcellular level, electron microscopy is often used to unravel the detailed structures, which are crucial for the motility machinery of unicellular organisms [6,14,21,23–27]. In combination with motility analysis, microscopy can also be used on mutant cell lines to further clarify the roles of various genes onto morphology and motility of unicellular parasites, as has been shown for trypanosomes [14,28–30] and apicomplexans [6,16,23,31].

Glossary

Deterministic lateral displacement (DLD): the displacement of flowing objects (e.g., cells or particles) along an array of pillars, depending on the size, shape, and deformability of the body. Therefore, the space between the pillars increases gradually perpendicular to the direction of the flow.
Friction coefficient: the viscous force $\vec{F}$ acting on a swimming cell, and the difference in velocity $\vec{v}$ between the cell and the surrounding medium, depend on the friction coefficient γ, which depends on the shape and size of the cell: $\vec{F} = \gamma\vec{v}$.
High-speed imaging: recording the movements of motile cells at more than 100 frames/s to elucidate even the finest details of their motility.
Mean squared displacement (MSD): from trajectories calculated area that a motile cell covers over time. Plotted in a log–log frame, the slope of the MSD reflects the diffusive behavior of the motile cell.
Microfluidics: the study of the dynamics of fluids (i.e., liquids and gasses) in devices with at least one dimension, usually width and/or height, in the order of micrometers.
Non-reciprocal movements: a series of motions, wherein multiple parts are moved in a specific order to cycle through fixed poses. Reversing the order of motions would result in a different series of poses.
Optical tweezers: using highly focused laser beams to manipulate cells and objects, without actually physically touching them.
Diffusive: a moving cell or particle, which changes the direction of its motion at random, exhibits diffusive behavior. The slope of the MSD derived from such a trajectory equals 1.
Subdiffusive: a cell which is confined to a small space, or that repeatedly bumps into a wall or becomes trapped, and exhibits a trajectory that crosses its own path repeatedly and thus covers less area compared to a randomly walking cell, is classified as sub-diffusional. The slope of the MSD derived from such a trajectory is less than 1.
Superdiffusive: a cell which is either passively dragged or actively swimming predominantly in one direction is classified as super-diffusional. It exhibits a trajectory nearly without crossing paths, and