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Abstract

Trypanosoma brucei is the causative pathogen for the fatal human disease African sleeping sickness. For over 160 years, cellular propulsion in *Trypanosoma brucei* has been considered to be in an auger-like motion and current understanding of the motility states the cell moves using left-helical waves which propagate along the flagellum. In contrast to the uniform flagellar beats laid out by the traditional model, we find that the frequency was lower at the posterior end compared to the anterior end, suggestive of an alternative and unique mechanism of motility exhibited by *Trypanosoma brucei.* Our new studies are significant in pioneering a new direction and providing important insight into the actual model of *Trypanosma brucei's* movement. As *Trypanosome* motility is central to disease pathogenesis, parasite development and disease transmission, this investigation provides a requisite step in efforts to exploit cell motility as a target for disease control in African sleeping sickness.

Introduction

Trypanosoma brucei uses flagellum movement to carry out disease pathogenesis, but the mechanism for carrying this out is not completely elucidated and understood. *T. brucei's* flagellum is an essential and critical component for the parasite to carry out cell motility, host-parasite interaction, cell division, and cell morphogenesis (Van Den Abbeele, J., 1999; Vickerman, K., 1988; Hill, K.L., 2003). Efforts to understand trypanosome pathogenesis and fundamental aspects of eukaryotic flagellum are directly related to understanding *T. brucei* flagellum.

The *T. brucei* development begins in the tsetse fly in what is referred to as a procyclic form. During this procyclic form, the necessity for its motility is particularly acute as the parasite completes a long migration from the midgut of the tsetse fly to the salivary glands. This migration is necessary for the *T. brucei* to complete its eventual transmission into its mammalian host. Though the path of the parasite's journey is known, there has yet to be a robust description for how it achieves this transport throughout the insect vector (Hill, K.L., 2003). Upon penetration inside the mammalian host that is achieved by a bite from the infected tsetse fly, the parasite adopts a bloodstream form, invading the central nervous system and rendering the host into a coma and ultimately, death. During all stages of its lifecycle, *T. brucei* resides extracellularly and thus is completely dependent upon its own cell motility for navigation within the insect vector as well as invasion into the central nervous system of the mammalian host. Such dependency that *T. brucei* has on its motility emphasizes the importance on studying the mechanism for its movement. For without an efficient method of propagation, the cell could not carry out its pathogenesis.

T. brucei is currently a devastating human and animal pathogen that causes significant human mortality and limits sustained economic development in sub-Saharan Africa (Welburn, D., 2002; Sternberg, J.M., 2004). The conventional and present model of the organism's motility, first described in 1843 (Gruby, D., 1843), assumes continuous auger-like rotation of the entire cell body driven by left helical waves propagating along the flagellum (Hill, K.L., 2003; Walker, P.J., 1961; Kohl, L. and Bastin, P., 2005). In an effort to understand and provide a detailed description of this motion, we performed quantitative analysis of the motility of *T. brucei* using differential-interference-contrast (DIC) microscopy. In doing so, we have discovered new information revealing an inconsistency in frequency beats down the cell body and taken numerous recordings of the cell's motility. Our findings provide important and valuable insight for progress towards targeting the cell motility as a novel means of therapeutic treatment. High-speed imaging to capture and analyze cell motility could be thus applied in efforts to understand other cell's forms of motion.

Materials and Methods

Trypanosome cell maintenance and motility assays

Procyclic 29-13 and BSF-SM cell lines were used throughout these experiments and maintained as described as follows. Procyclic cells were

maintained in Cunningham's SM semi-defined maintenance media supplemented with 10% heatinactivated fetal calf sera, 5µg/ml G418 (Gibco) and 50µg/ml Hygromycin (Gibco). Bloodstream BSF-SM cell were cultured in HMI-9 media supplemented with 15% heat inactivated fetal calf sera and 15µg/ml G418 (Gibco). Cell growth was monitored using a Z1 Coulter Particle Counter (Beckman Coulter, USA). For motility assays, cells were taken from mid-logarithmic phase cultures and loaded onto poly-L-glutamate treated slides (Gadelha et al. 2005). Once loaded on the slides, cells were visualized using a differential-interference-contrast microscope (Nikon TE2000U) with a 150X magnification, and cell movements were captured using an Ultima SA1 High Speed Video System (Photron USA, Inc.). Procyclic cell motility was assayed at room temperature, while bloodstream cell motility was assayed on a stage heated at 37°C. In either case, cells were not assayed for more than 30 minutes following loading of the cells onto the slide chamber.

Image Acquisition

Image sequences of up to 3 seconds was recorded at 1 kHz and analyzed to elucidate the fundamental features of unrestricted cell motion. To avoid influencing the observed motility, cells were allowed to propagate in three dimensions. All image acquisitions were performed using the image analysis program, NIH-ImageJ (http://rsbweb.nih.gov/ij). To allow for consistent analysis of moving cells, only cells that swam in a direct path for the duration of 1 or more seconds were considered for analysis. Examples exhibiting vibration or other motions derived from ambient effects were excluded from the analysis.

Denoising Algorithm

A high signal-to-noise ratio was established by optimally enhancing image contrast by NIH-ImageJ and Matlab (Mathworks Inc.) Parameters were determined including the dimensions to perform the convolution and pixel intensity values to threshold the contrast.

Center of Mass (COM) Algorithm

To denoise images, we implemented a center-of-mass (COM) algorithm written in Matlab that set all pixel values less than the desired parameter equal to zero, eliminating hot pixels that might affect the COM algorithm later. Each pixel value was weighted from their position and the summation of those values was divided by the summation of all the pixel values to arrive at the COM. This position was then tracked over time throughout all the image frames.

Frequency Determination

The movie sequence was run through the denoising algorithm and COM of algorithm in Matlab. For frequency determination of the trypanosome section, the COM for each section was then plotted out and the number of peaks counted and divided by the time interval of the movie to derive the frequency. The standard deviation was the differences in time between each peak.

Results

Observation of Motility

To investigate the motility of *T. brucei,* millisecond imaging of both procyclic and bloodstream cells was acquired (Figure 1). Higher speeds of acquisition were tried as well, up to 10,000 frames per second, but the millisecond resolution was sufficient enough for capturing the motion of the cell. High flagellar movement was observed in both forms with motion in bloodstream cells more rapid and

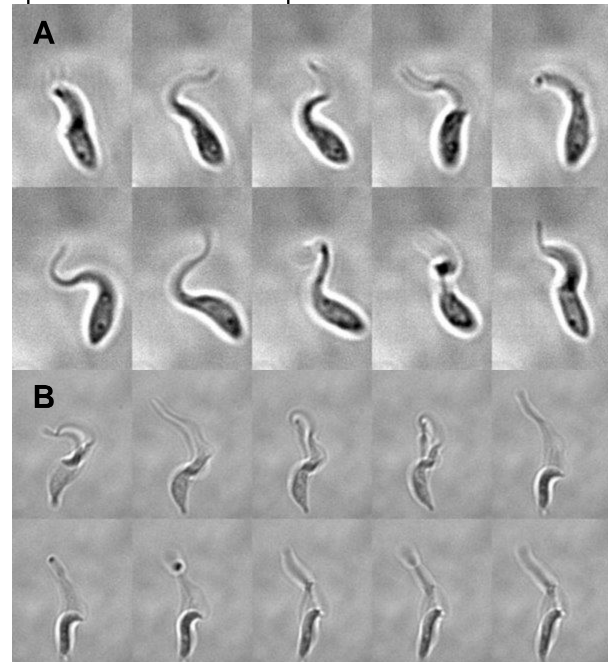

Figure 1. Millisecond imaging of (A) procyclic and (B) bloodstream cell motility.
(A) The top image sequence illustrates the motility of a procyclic cell at 30 millisec intervals from left to right taken at 1 kHz
(B) The bottom image sequence illustrates the motility of a bloodstream cell at 30 millisec intervals from left to right taken at 1 kHz

irregular than that observed in procyclic cells, but still maintaining the fundamental features of forward motion defined by these cells.

The cell body was then segmented off in equal lengths and the center-of-mass (COM) position movement of each section were tracked over time to show the differences in motion between the anterior and posterior of the cell (Figure 2). We observed that the anterior end or tip of the cell had a more pronounced movement

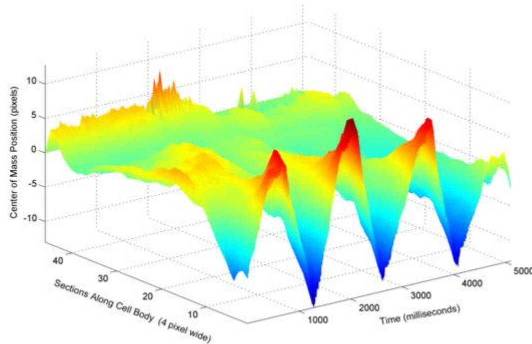

Figure 2. Center of Mass Tracking of Cell Movement Over Time
Segments of the cell were sectioned off in 4 pixel slices and the center of mass of each segment was tracked over time. Here one procyclic in 10% fetal bovine serum is shown. The x-axis shows sections along the cell body in 4 pixel increments, y-axis shows the time in milliseconds, z-axis is the COM position of the each cell body section.

and seemingly higher frequency movement than the posterior end or base of the cell in both its bloodstream and procyclic forms.

Quantification of Frequency

Since the image sequences and center of position trackings suggested that the beat frequency at the anterior of the cell is different than at its posterior end, we sought to analyze the frequency for each segment along the cell body. Using the COM algorithm, each cell was divided in to equal sections and their frequency determined (Figure 3). The average frequency determined for each segment along the cell body was considerably higher at the anterior end of the cell, around 17 Hz, than the average base frequency, approximately 7 Hz. Previous literature on the analysis of *T. brucei* movement have only measured velocity, moving as fast as 20 μm/s and described the parasite's motion qualitatively including it's unusual tip-to-base wave propagation and seemingly spiral motility (Hill, K.L. 2003). This lack of quantitative characterization of *T. brucei's* motility is largely due to the cells elusive, erratic motion as well as 3-dimensional movement, which posed problems for robust analysis. Our

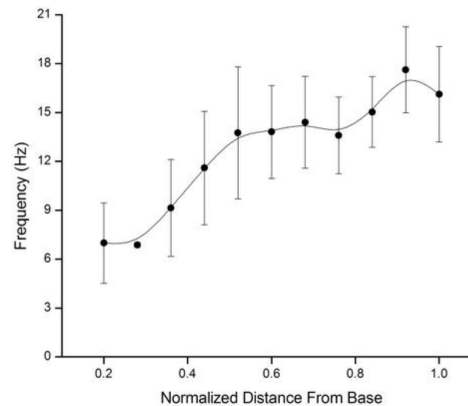

Figure 3. Frequency of a procyclic cell in 10% fetal bovine serum
Frequency of a procyclic cell sections showing the beat frequency (Hertz) along the length of the cell body from base to tip. Each cell length was normalized to 1 and segmented in 4 pixels or approximately 0.5 micrometers.

quantitative analysis, which accounts for these issues, produced new and unexpected findings for the *T. brucei's* beat frequency.

Discussion

Traditionally the *T. brucei* flagellum is generally considered to wrap in a left-hand helix around the cell body and exhibit auger-like rotation of entire cell body (Ginger, M.L. et al., 2008; Moreira-Leite, F.F. et al, 2001; Ralston, K.S. and Hill, K.L., 2008). However, our study, detailing a quantitative analysis of motility of *T. brucei*; suggests that *T. Brucei* does not follow this conventional motion (Figure 1). The seemingly decreasing frequency from the anterior end of the cell towards the posterior was confirmed by analysis of COM position (Figure 2; n=20) and frequency determination of individual procyclic cells (Figure 3; n=10). These findings implied that an external dissipating force is required to release energy in order to lower the beat frequency and would not be consistent with the traditional model. Furthermore, the observations presented here were never before noticed in *T. Brucei* and present a new paradigm for cell motility exhibited by the parasite.

According to the previous model, there should be no dissipation of frequency. Nonetheless, after COM trackings and high speed analysis, the procyclic forms of the cell clearly conveyed a discrepancy in frequency throughout

the body of the cell, implying that a new mechanism for motility must be at present.

Trypanosome motility is central to parasite development and pathogenesis as migration of the parasite from the tsetse fly midgut to salivary glands is required for disease transmission, and parasite traversal of the human blood brain barrier marks the onset of lethal sleeping sickness (Ginger, M.L. et al., 2008; Hill, K.L., 2003; Van Den Abbeele, J. et al, 1999; Mulenga, C. et al., 2001; Engstler, M. et al., 2007). *T. brucei* depends upon its own flagellum for motility in both hosts, and perturbation of the flagellar apparatus is often lethal (Broadhead, R. et al., 2006; Ralston, K.S. and Hill, K. L., 2006). Previously, the mechanism behind *T. brucei's* movement was thought to be a simplistic left-handed helical motion with constant beat frequencies propagating down the length of the body. However, our findings uproot this model: instead of constant beat frequencies in the wave; *T. brucei*, in fact, has an irregular beat frequency. By using novel analysis techniques, including COM algorithms and high-speed image acquisition, our studies suggest there must be an alternative modality for its motion to explain the inconsistent frequencies of wave propagation. This initial discovery could provide an important prerequisite towards understanding the pathology of the parasite.

These new findings are important towards understanding properties ascribed to the trypanosome motility and mechanism behind the paraflagellar rod which gives the *T. brucei* flagellum its shape (Bastin, P. et al, 1998; Bastin, P. et al, 2000). Since the flagellum is attached along the length of the cell body via the paraflagellar rod; the knowledge that it is capable of producing irregular wave beat propagations could prove vital to understanding its properties.

By establishing a quantitative foundation for molecular studies of *T. brucei* flagellar motility, here we present a novel finding to exploit the potential of the flagellum as a target for therapeutic intervention in African sleeping sickness. Since the flagellum is responsible for *T. brucei's* motion and consequently its pathology; if the mechanism and properties behind this motility could be understood, one possible therapeutic treatment would be to inhibit its motion and thus, prevent the onset of the disease. Further studies to characterize *T. brucei's* motion and mechanism of motility could additionally reveal new insights for the modalities of motion behind other flagellated species or parasites that invade the bloodstream and migrate to similar tissues. As there has yet to be a model to describe irregular wave propagations along *T. brucei's* body; there is also the strong possibility of a novel motility present which has not been observed or paralleled in other flagellated cells. Furthermore, our DIC microscopy was effective at capturing the motion of the *T. brucei* flagellar at up to 1000 frames per second, suggesting that high speed analysis is broadly applicable for discovering novel features of flagellar motility in other organisms (Bray D., 2001; Ginger, M.L. et al., 2008; Berg, H.C., 2003).

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