MODE SWITCHING AND SYNCHRONIZATION IN *CHLAMYDOMONAS* FLAGELLA

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Summary Cilia and flagella are implicated in a variety of physiological processes, ranging from sensory transduction to provision of cellular motility. Using the model unicellular alga *C. reinhardtii*, whose flagella typify the evolutionarily conserved eukaryotic flagellar structure, we probe the permissible modes of beating that a flagellum can exhibit, and in turn how a pair of them can interact, with the aim of furthering an understanding of the generic phenomenon of how neighbouring, weakly coupled flagella can be brought into synchrony by virtue of their immersion in a shared fluid environment. The circumstances affecting how such synchrony can arise, and in particular, where synchrony can fail, or take unusual forms, may be of great biomedical consequence in the context of mammalian ciliopathies.

INTRODUCTION

Implicated in a variety of important life processes, interchangeably termed cilia or flagella, eukaryotic ciliary appendages display a fascinating multitude of individual as well as collective motions that calls for a much deeper understanding than that is currently available. Despite their ubiquity, the eukaryotic flagellum is a remarkably conserved structure: comprising of 9 microtubule doublets arranged on the periphery, and attached through a complex system of radial spokes and regulatory proteins, to a central pair of microtubules which also extends throughout the length of the flagellum. In most cases, the precise and coordinated action of molecular motor dyneins culminates in the periodic asymmetric beat of the flagellum - alternating between power and recovery strokes in a manner frequently adopted by low Reynolds number microswimmers. Extracting time series data from our high-speed movies we have modelled beating dynamics of the flagella pair in the alga *Chlamydomonas reinhardtii* (CR), as noisy coupled oscillators which phase synchronize during normal breaststroke beating. Detailed analysis of beating dynamics reveals the existence of alternate states of higher frequencies, as evidenced by dynamics ranging from transient phase slips in the wild-type, to a novel mode of anti-phasic synchrony displayed by a CR mutant [4], and to a high-frequency undulatory motion that defines the flagellar "photoshock" responses. This ability to alter flagellar waveform when induced by strong light or mechanical stimuli is not unique to *Chlamydomonas* flagella, and can be found in typical avoidance responses of organisms such as paramecium [1]. Our results appear to acknowledge a range of permissible dynamical states for concerted motor action, which are sequentially attained through a mechanism thought to be mediated by calcium-binding proteins. Here, by inducing the photoshock response in a uni-flagellated mutant of *C.reinhardtii* we examine the ensuing recovery transition from undulatory back to breaststoke beating undergone by its single flagellum. Such a transition exemplifies the Ca^{2+}-sensitive sequential decrease of beat frequency, and concomitant re-emergence of asymmetry in the flagellar waveform.

MATERIALS AND METHODS

To obtain identical cells for each *C. reinhardtii* strain chosen for analysis, cells were first streaked out onto agar plates. Colonies were then selected and transferred to liquid culture medium (Tris-Acetate Phosphate). Liquid sub-cultures were grown photo-heterotrophically with the acetate from TAP serving as a carbon source, with constant shaking, maintained in growth chambers (Binder, Germany) set at $24°C$, and a daily light-dark (LD) cycle of $14 : 10$. In order to ensure maximal cell viability and culture synchrony, cell samples for purposes of experimentation were extracted from the second liquid sub-culture onwards, and at exponential growth phase ($\sim 10^5$ cells/ml).

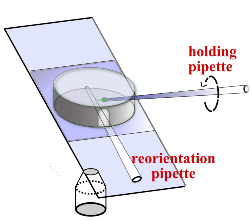

Figure 1: *set up*

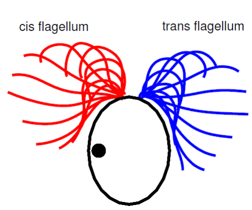

Figure 2: *a single beat*

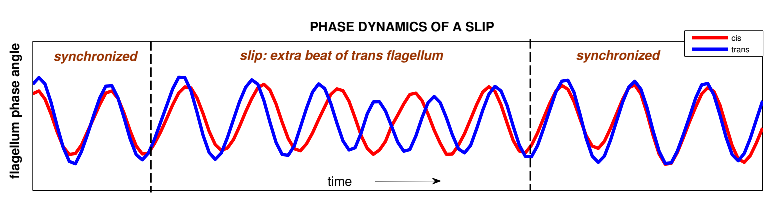

Figure 3: a *slip*: *note transient decay in waveform amplitude*

Visualization of flagellar dynamics under bright field illumination on a Nikon TE2000-U inverted microscope is achieved through attachment of individual cells via the posterior end through gentle suction provided by a micropipette (shown). Holding and re-orientation micropipettes are attached to orthogonally positioned micromanipulator arms, thereby maximizing freedom for repositioning held cells. High speed movies were recorded at up to 1000fps and image-processed to reveal flagella motion. Processing and analysis algorithms were implemented through custom MATLAB routines.

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RESULTS

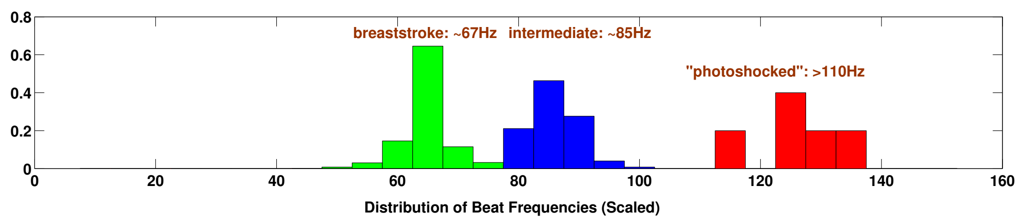
A simple coupled oscillator model

Previously, characterization of long-time series breaststroke swimming in *wt Chlamydomonas* led to a model of biflagellar dynamics consistent with coupling through hydrodynamic interactions [2]. Conforming to a description of each flagellum as an oscillator equipped with a time-dependent phase variable (θ_1 and θ_2), observations of transient episodes of departures from the base, phase-synchronized state - termed *slips* (see Fig.3), were successfully explained upon incorporating a stochastic component into the dynamics. Now the two flagella of each CR cell (distinguished by their relative displacement from the single eyespot, and dissimiliar in ontogenetic age), have inherently different frequencies, this difference we shall denote by $\delta\nu$. The origin of stochasticity arises not only from the non-identical nature of the two flagella, but also from internal, biochemical noise, as well as from external sources, such as photonic noise. The phase difference $\Delta = \theta_1 - \theta_2$ evolves in a manner captured by the stochastic Adler equation $\dot{\Delta} = \delta\nu - 2\pi\epsilon\sin(2\pi\Delta) + \xi(t)$, where ϵ is the coupling magnitude. The Gaussian white noise term $\xi(t)$ satisfying $\langle\xi\rangle = 0$ and $\big\langle\xi(t)\xi(s)\big\rangle = \kappa\delta(t-s)$, enforces stochasticity. Noise-induced phase-slippage events can thus be interpreted in terms of an over-damped particle hopping between neighbouring quasi-stable minima, on the tilted-washboard potential corresponding to the equation for Δ. Sufficiently high noise can result in phase-diffusion down this potential, whose tilt is dictated by $\delta\nu$, which is manifest in the beating cell as a third state termed the *drift* (see iii for further discussion).

What's all the noise about?

Flagellar axoneme activity is precisely generated and moderated by motor dyneins, in a manner as yet unclear but thought to be attributed, at least in part, to spontaneous beating emerging from dynamical instabilities. While the above coupled dynamical states predicted by the theory are indeed exhibited by our micropipette-held cells, how does the living flagellum, an entity distributively-attenuated by axonemal dyneins, obtain and respond to the cues required to accordingly alter its beating mode? Intracellular ions, such as *calcium*, may function through direct action on individual dyneins, as an order parameter to tune flagella bending. Thus, we seek to address the question of how pairs of flagella synchronize by first isolating the noise and dynamics intrinsic within a single flagellum.

A uni-flagellar photoshock



Capturing the photoshock response in uni-flagellated mutants of *Chlamydomonas* (strain CC-1926, Chlamy Center), we probe the flagellum waveform changes accompanying the gradual decay of the initial influx of calcium upon stimulus-induced membrane depolarisation [5]. Preliminary results produce distributions of beat periods which reveal coalescence at discrete frequencies beyond that of normal beating.

CONCLUSIONS

Improving experimental and image processing techniques we have succeeded in quantifying in much greater detail than before the flagellum beat of *Chlamydomonas*. Exploiting the flagellar photoshock response our preliminary tracking of the range of waveforms of the uni-flagellar beat appears to indicate that coordinated motor dynein action may result in *discrete* modes of permissible beating of the flagellum. This notion is further corroborated by concurrent work regarding a phototaxis mutant of *Chlamydomonas* whose two flagella displays a form of anti-phase synchronization, which, in comparison with the normal biflagellar breaststroke, is characterized by a distinct waveform and higher beating frequency [4].

References

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