Noise and Synchronization in Pairs of Beating Eukaryotic Flagella

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It has long been conjectured that hydrodynamic interactions between beating eukaryotic flagella underlie their ubiquitous forms of synchronization; yet there has been no experimental test of this connection. The biflagellate alga *Chlamydomonas* is a simple model for such studies, as its two flagella are representative of those most commonly found in eukaryotes. Using micromanipulation and high-speed imaging, we show that the flagella of a *C. reinhardtii* cell present periods of synchronization interrupted by phase slips. The dynamics of slips and the statistics of phase-locked intervals are consistent with a low-dimensional stochastic model of hydrodynamically coupled oscillators, with a noise amplitude set by the intrinsic fluctuations of single flagellar beats.

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One of the truly remarkable discoveries in biology is a connection between the process that determines the leftward position of the heart in a human body and the swimming of microscopic green algae. The link is provided by flagella [1,2], the microscopic appendages whose beating propels algae [3] and which also set up circulating flows in a developing embryo that establish the left-right asymmetry of its body plan [4,5]. Flagella play an important role in many aspects of life, from fluid transport in the reproductive system and the respiratory tract, to mechanical and biochemical signal transduction in the kidneys and eyes. The coordinated motion of groups of flagella, from pairs to thousands, is often crucial to the successful performance of these functions [6].

Observations of nearby sperm flagella beating in phase led Taylor [7] to consider the simplified problem of two nearby waving sheets in Stokes flow. He found the intuitive result that viscous dissipation is minimized for a vanishing phase shift between the two imposed waves of displacement. However, this model does not provide a mechanism by which bodies with slightly different frequencies, starting at an arbitrary initial condition, would evolve to synchrony, a fundamental requirement for the robust synchronization observed *in vivo*. Within a phenomenological description of active bending moments, Machin [8] showed that a coupled pair of flagella can indeed synchronize; while the mechanochemical details of synchronization are still under active investigation, the emerging theoretical consensus is that hydrodynamic interactions are indeed the root cause [9–13].

Tests of theories of synchronization require model organisms capable of precise visualization, with broadly representative biology. It has become clear [6] that the biflagellate alga *Chlamydomonas reinhardtii* [Fig. 1(a)] is ideally suited to these studies. *C. reinhardtii* has a spheroidal body $\sim 5\ \mu$m in radius and a pair of flagella $\sim 12\ \mu$m long, extending from one end. The two flagella are termed *cis* and *trans* based on their location with respect to the eye spot, a primitive photosensitive organelle. They typically beat at ~ 50 Hz in a synchronous breaststroke with a small phase difference, and this dynamics is regulated by the cell to move to areas with optimal levels of light and essential chemicals, and to avoid sinking. Kamiya and Hasegawa [14] showed that the two flagella of demembranated *C. reinhardtii* "cell models" have distinct beat frequencies. Although cells so treated may not be fully representative of the native form [15], these findings suggest that in living cells, some mechanism must be in place to ensure synchronous beating. At the same time, Rüffer and Nultsch [16] used short (~ 2 s) high-speed movies to show tantalizing evidence of a complex dynamics of beating flagella that is not captured by the picture of synchronous breaststrokes.

Here, we explore the complex breaststroke beating in detail, using high-speed video microscopy to analyze long times series containing tens of thousands of beats. Our results show that *C. reinhardtii* flagella pairs exhibit noisy synchronization interrupted by phase slips. Well known in the study of coupled oscillators [17], this *phase diffusion* in

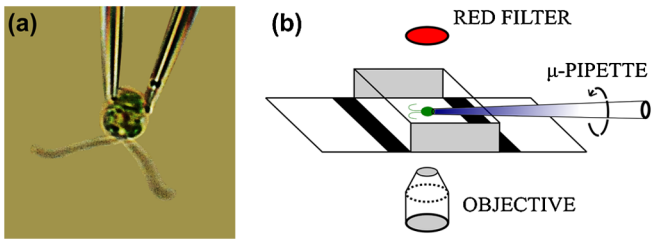

FIG. 1 (color online). Experimental system. (a) A single cell of *Chlamydomonas reinhardtii* held on a micropipette. (b) Schematic of apparatus for visualization and micromanipulation of cells.

eukaryotic flagella has not been anticipated by theory. Despite the complexity of this system, we find consistency with a low-dimensional model of coupled noisy phase oscillators and a coupling strength consistent with hydrodynamic interactions. These results show for the first time the role of biochemical noise in the dynamics of eukaryotic flagella.

C. reinhardtii (UTEX 89 [18]) was grown axenically in standard *Volvox* medium (SVM) [19] with sterile air bubbling, in growth chambers (Binder, Germany) set to a cycle of 16 h in cool white light (~4000 lux) at 28 °C and 8 h in the dark at 26 °C. Cells were harvested in a light period within their exponential growth phase (~10^6 cells/ml) and immediately transferred to the sample cell [Fig. 1(b)], where observation started after 30 min to allow for acclimatization. Individual cells were held by gentle suction provided by a manual microinjector (Sutter Instrument Co., USA) connected to micropipettes with 2–4 μm diameter tip, hosted in pipette holders (World Precision Instruments, USA). Pipettes were prepared with a puller (P-97, Sutter) and reshaped with a microforge (DMF1000-2, WPI). The holders were mounted on custom stages providing free rotation around the pipette axis, and fit on motorized micromanipulators (Patchstar, Scientifica, UK). This setup allows precise positioning and reorientation of the captured cells for optimum visualization. We used bright field illumination on a Nikon TE2000-U inverted microscope with a Nikon Plan Fluor ELWD 40× objective, which allows imaging of cells further than 1 mm from the chamber's surfaces, minimizing wall-induced hydrodynamic effects on the flagella. A long pass interference filter with a 10 nm transition ramp centered at 620 nm (Knight Optical, UK) was used to avoid any phototactic response [20]. Movies up to 3 min long were captured by a high-speed video camera (Phantom V.1, Vision Research, USA) at 500 fps, and transferred to disk for processing and analysis with custom MATLAB routines.

Synchronization and phase slips are best viewed normal to the plane containing the two flagella (Fig. 2). Each passage of the left (L) or right (R) flagellum across a small interrogation region corresponds to a peak in the intensity signal $X_{L,R}(t)$, with consecutive peaks separated by a full beating cycle. We characterize this periodic motion with a phase $\theta_{L,R}(t)$, which advances by 1 between successive peaks, with intermediate values estimated by linear interpolation. The phase difference, $\Delta(t) = \theta_L(t) - \theta_R(t)$, will then fluctuate around a constant value during synchrony [21]. Occasionally, one of the two flagella accumulates an extra beat and $\Delta(t)$ slips by one unit [Figs. 2(a)–2(l)]. The time series in Figs. 2(m) and 2(n) show further that the single extra cycle of a phase slip is completed in ~15 beat periods or ~0.3 s. This beating dynamics is characteristic of freely swimming cells as well [16]. Results from 21 different individuals observed over a much longer time scale reveal that the same organism can have slips of either

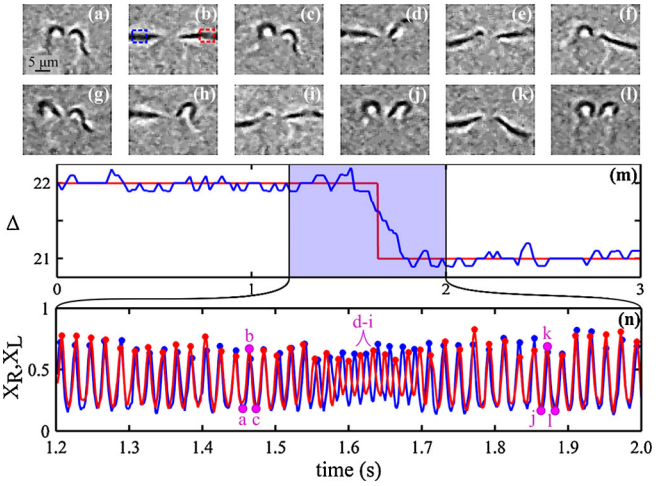

FIG. 2 (color online). A phase slip in the beating of *C. reinhardtii* flagella. (a)–(l) Individual image-processed movie frames at times indicated in panel *n*. (m) Phase difference $\Delta(t)$ between flagella as a function of time. (n) Extracted signals from the interrogation areas [seen in (b)] near each of the two flagella.

sign [Fig. 3(a)], and that the occurrence of these events is not regular, but rather is characterized by a wide distribution of waiting times [Fig. 3(b)]. Stochasticity clearly plays an important role in the observed dynamics.

We model the synchronized flagella pair as two phase oscillators, $\theta_{L,R}$, evolving with distinct intrinsic natural frequencies, $\nu_{L,R}$, and coupled through an antisymmetric function of the phase difference,

$$\dot{\theta}_i = \nu_i + \pi\epsilon \sin[2\pi(\theta_j - \theta_i)] + \xi_i(t), \qquad (1)$$

where $i, j \in \{L, R\}$. The Gaussian white noise ξ_i, with $\langle \xi_i(t) \rangle = 0$ and $\langle \xi_i(t)\xi_j(t') \rangle = T_{\text{eff}}\delta(t - t')\delta_{i,j}$, is a surrogate for noise in a single flagellum's dynamics. These equations reduce to a stochastic Adler equation [17],

$$\dot{\Delta} = \delta\nu - 2\pi\epsilon \sin(2\pi\Delta) + \xi(t), \qquad (2)$$

where $\delta\nu = \nu_L - \nu_R$, and $\xi = \xi_L - \xi_R$ satisfies $\langle \xi(t)\xi(t') \rangle = 2T_{\text{eff}}\delta(t - t')$. Equation (2) describes the overdamped motion of a particle diffusing on a tilted washboard potential $V(\Delta) = -\delta\nu\Delta - \epsilon\cos(2\pi\Delta)$ [Fig. 3(a), inset] [22]. In this model, the noisy dynamics during synchronization is represented by fluctuations around local minima in V, with the autocorrelation of Δ easily shown to decay as $C(t) = C_0 \exp(-t/\tau_c)$. This is indeed observed experimentally (Fig. 4). Noise-induced hopping events bring the system over to adjacent minima and represent phase slips. The presence of a bias $\delta\nu$ implies that the ratio of forward to backward jumping probabilities P_+/P_- is not unity. For each experiment, we used these three observables (C_0, τ_c, P_+/P_-) to estimate the three parameters of the stochastic model, $T_{\text{eff}} = C_0/\tau_c$, $\delta\nu = T_{\text{eff}} \log(P_+/P_-)$, $\epsilon = 1/2\pi\sqrt{\delta\nu^2 + 1/(2\pi\tau_c)^2}$ [23]. From analysis of all available data in the synchronized

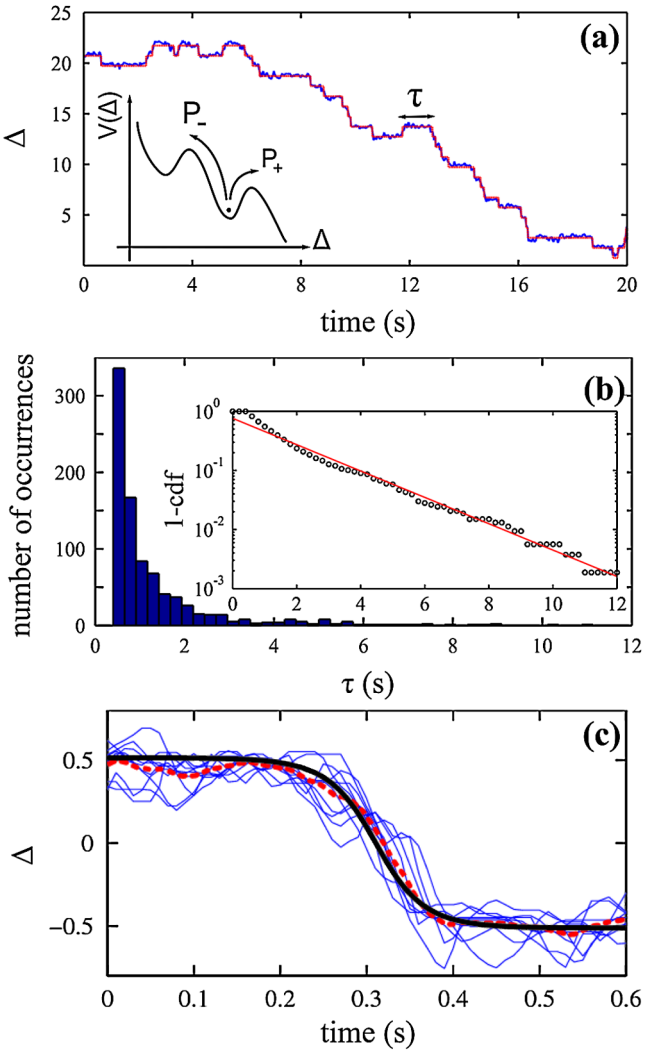

FIG. 3 (color online). Dynamics and statistics of phase slips. (a) Long time series of $\Delta(t)$ illustrating forward and backward slips. Inset: sketch of the tilted washboard potential used in the model. (b) Probability distribution of synchronization times and its cumulative distribution function (cdf) showing approximate exponential decay. (c) $\Delta(t)$ for 10 slips events, the average slip (dashed line) and the integrated trajectory from the deterministic part of the model (solid line) for $\delta\nu/\bar{\nu} = 0.004$, $\bar{\nu} = 47$ Hz, and $\epsilon/\bar{\nu} = 0.015$; $\tau_{\text{slip}} \sim 0.22$ s.

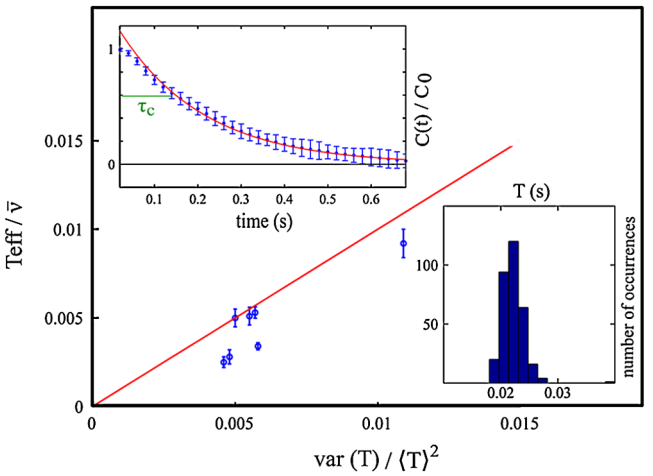

FIG. 4 (color online). Comparison of single flagellum noise levels before (y axis) and after (x axis) partial deflagellation. In the latter case, the noise can be extracted from the distribution of beating periods (lower inset), and compared to the value estimated from the autocorrelation of the coupled dynamics (upper inset). Only experiments in which the isolated flagellum had a long period of uniform behavior are shown (7 out of 14).

regime, we find $\langle T_{\text{eff}}/\bar{\nu}\rangle = 0.006$ (with a range 0.002–0.012), $\langle \delta\nu/\bar{\nu}\rangle = 0.008$ (0.001–0.02) and $\langle \epsilon/\bar{\nu}\rangle = 0.008$ (0.003–0.015), and $\langle \bar{\nu}\rangle = 50.6$ Hz. The large bending rigidity of the flagellar axoneme, $\kappa \simeq 4 \times 10^{-22}$ N m^2, implies that thermally driven fluctuations are smaller than 10 nm, well below our experimental resolution. Their contribution to the measured observables, in particular, the noise strength, is therefore negligible.

An estimate of the hydrodynamic coupling between two idealized flagella [24] is $\epsilon_{\text{est}} \simeq \rho\tau_r\nu_R\nu_L$. Here, τ_r is a time scale for decay of flagellar displacements away from the unperturbed periodic cycle. The validity of a model of coupled phases like (2) rests in part on this relaxation time being much shorter than the flagellar beat period, $1/\bar{\nu} \sim 20$ ms. In the limit of small perturbations, τ_r can be estimated from the viscosity η of the surrounding fluid, the flagellar bending rigidity κ, width a, and length l as $\tau_r = 3\pi\eta a l^3/\kappa \sim 5$ ms, thus satisfying the above requirement. The prefactor ρ depends on the density of the flagellar arrangement [24]; in our experiments, $\rho = 0.025$. These values yield a coupling strength $\epsilon_{\text{est}}/\bar{\nu} \sim 0.006$, which compares very well with the experimental results. This is a strong indication of the importance of interflagellar hydrodynamic interactions. Either biochemical or mechanical coupling through the cell wall or the cytoskeleton could very well play an important role, but hydrodynamic coupling alone would be sufficient to induce the observed synchronization.

Despite the coupling, during synchrony the two flagella carry a signature of their *intrinsic* frequency difference, in that Eq. (2) predicts the existence of a small *phase-lag* between the two flagella, given by the fractional part of the value of the potential minima, $\Delta_0 = \arcsin(\delta\nu/2\pi\epsilon)/2\pi$. Indeed, the largest computed value of $\simeq 1/11$th of a cycle is in excellent quantitative agreement with previous observations [16]. From the average values of the parameters of the model, it is also possible ([22], Sec. 7) to predict the average duration of a synchronized period to be $\langle\tau\rangle_{\text{est}} = 2.0$ s, which agrees well with the value extracted from the distribution function of all synchronous periods from the 21 experiments, $\langle\tau\rangle_{\text{exp}} = 1.92$ s [Fig. 3(b)]. Synchrony is interrupted by slips whose duration is also consistent with the model. A series expansion of the potential $V(\Delta)$ predicts exponential growth of small perturbations near the

unstable maxima with a time scale $\tau_{\mathrm{slip}} \simeq 1/2\pi\epsilon \sim 0.2$–$1.0$ s, consistent with our observations of slips' durations. Indeed, in all experiments, the evolution from a maximum to an adjacent minimum can be determined by integration using the estimated parameters and shows very good agreement with the average slip along the entire trajectory [Fig. 3(c)].

These observations suggest the need to characterize the beating of *individual* flagella. While the precise mechanism coordinating the activity of axonemal motors is still unknown [11–13], the very molecular nature of the motors, with their associated reaction rates and attachment probabilities, suggests that the beating process is intrinsically stochastic. However, in contrast to our advanced understanding of noise in the rotary motor of bacterial flagella [25], our knowledge of eukaryotic flagellar noise is poor. As a first step, we used a second pipette to remove one flagellum to study the other in isolation, starting immediately after removal to minimize the influence of natural intracellular processes leading to flagellar regrowth [26]. When successful, this crude process leaves the other flagellum beating at a reasonably uniform frequency, and offers the opportunity to study the isolated dynamics of a flagellum just after having observed its coupled behavior. The noise level of an isolated flagellum can be extracted from the mean and variance of the distribution of beating periods as $T_{\mathrm{eff}}/\bar{\nu} = \mathrm{var}(T)/\langle T\rangle^2$. The results (Fig. 4) support the inference that the coupled dynamics' noise comes mainly from that intrinsic to the individual flagella, although other contributions cannot be ruled out.

We have shown that pairs of beating eukaryotic flagella exhibit noisy synchronization described by a simple phase oscillator model, with a coupling consistent in magnitude with that expected from hydrodynamic interactions. Despite its simplicity, the model successfully predicts interflagellar phase-lag during synchrony, the average length of a synchronous period, the average trajectory during a slip event, and it is consistent with results from single flagella. Elsewhere, we report the observation that occasionally the two flagella beat at different frequencies for extended periods and its relation to the run and tumble-like swimming of *C. reinhardtii* [23]. Issues for further experimental study include the biological importance of slips, the role of flagella length on synchronization, and simultaneous imaging of synchronous beating and intraflagellar calcium dynamics [27]. One of the main theoretical challenges is to explain the origin and magnitude of the observed noise.

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