Antiphase Synchronization in a Flagellar-Dominance Mutant of Chlamydomonas

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Groups of beating flagella or cilia often synchronize so that neighboring filaments have identical frequencies and phases. A prime example is provided by the unicellular biflagellate Chlamydomonas reinhardtii, which typically displays synchronous in-phase beating in a low-Reynolds number version of breaststroke swimming. We report the discovery that ptx1, a flagellar dominance mutant of C. reinhardtii, can exhibit synchronization in precise antiphase, as in the freestyle swimming stroke. High-speed imaging shows that ptx1 flagella switch stochastically between in-phase and antiphase states, and that the latter has a distinct waveform and significantly higher frequency, both of which are strikingly similar to those found during phase slips that stochastically interrupt in-phase beating of the wild type. Possible mechanisms underlying these observations are discussed.

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Living creatures capable of motion seldom restrict themselves to a single mode of propulsion. Pairs of appendages of multilegged organisms can be actuated synchronously in-phase, out-of-phase, or asynchronously by a “central pattern generator”[1]. In the world of aquatic microorganisms, where there is no central nervous system, the cilia and flagella adorning algae and bacteria are the “limbs” which exhibit various synchronization modes, generating swimming [2]. Within a given eukaryotic organism, the motor-driven undulations of flagella can be found to synchronize in two stereotypical ways. Biflagellates epitomized by the alga Chlamydomonas [3] display synchronous beating with identical frequencies and phases [4, 5]. Those with multitudes of cilia/flagella, such as unicellular Paramecium [6] or multicellular Volvox [7], exhibit metachronal waves in which flagellar phases vary monotonically with position. Theory [8–10] suggests that these modes of synchronization can arise from fluid dynamical coupling between flagella, possibly assisted by waveform compliance.

Flagellar synchronization is more complex than the simplest models of coupled oscillators would suggest; beating is intrinsically stochastic, cells can switch between synchrony and asynchrony [5], and flagella within a single organism can be functionally distinct. These features are well-established for Chlamydomonas; the flagella of wild-type (wt) cells typically exhibit a noisy in-phase (IP) breaststroke (Fig. 1a). Terned cis and trans for their proximity to the cell’s eyespot, the two flagella are differentially affected by calcium, exhibiting a tunable flagellar dominance [11] important in phototaxis.

We report here an alternative mode of synchronization not previously quantified [12] in eukaryotes, in which flagella lock in antiphase (AP) synchronization. For a range of conditions [13], this behavior can be sustained in time by the “flagellar-dominance” mutant ptx1 of C. reinhardtii [14]. While ptx1 cells exhibit no gross motility defects, they have defective phototaxis [12, 14, 15] thought to arise from lack of Ca2+-dependent flagellar dominance. We discuss mechanisms proposed for AP synchronization [8, 16–19], and suggest that our observations support active filament models [20] which exhibit discrete undulating modes of flagella.

Wild-type (CC125) and ptx1 (CC2894) strains [21] were grown photo-autotrophically in Tris-Minimal medium [22] with revised trace elements [23] and air bubbling in a diurnal growth chamber at 24°C on a 14:10 h light-dark cycle with a light intensity of 90 µE m−2 s−1 [5]. Cells were harvested from 1 or 2 day-old cultures at a density ~ 6 x 10^5 cells/ml, during hours 4 and 5 of the day, washed with buffer HKC-40/10 [24] and allowed to regrow flagella for at least 2 hours. Cylindri-

![Waveforms of C. reinhardtii. Logarithmically-scaled residence time plots averaged over O(10^6) beats overlaid by waveforms, color-coded in time. The wt displays IP breaststroke beating (a) stochastically interrupted by phase slips (b) in which one flagellum (here, trans) beats faster with an attenuated waveform. ptx1 displays an IP state (c) nearly identical to the wild-type (a), and a high-frequency AP state (d). Large and small ovals indicate cell body and eyespot, respectively.](image-url)
FIG. 2. (color online). Beating dynamics. (a) Phase difference $\Delta = (\psi_{\text{trans}} - \psi_{\text{cis}})/2\pi$ showing half-integer jumps between IP and AP states. Insets show waveforms in the two states. (b) Instantaneous frequencies of AP and IP states. (c) Distribution of instantaneous frequencies during IP beating and of the faster flagellum during slips, across all sampled wt cells. (d) IP and AP instantaneous frequency distributions, across all sampled ptx1 cells.

There are four key observations. First is the existence of the AP state itself (Fig. 1d), visualized by discrete waveforms within one cycle, color-coded in time and overlaid on a spatial map of average flagellar waveforms within one cycle, color-coded in time. Video microscopy was performed at 1,000 fps (Fastcam SA3, Photron, USA), post-processed in MATLAB. After each recording the filter was removed to locate the orange-colored eyespot and identify the cis and trans flagella. Experiments with wt cells showed that Chlamydomonas need to be acclimated for $\geq 20 - 30$ min before characteristic synchronized breaststrokes are observed [4, 5]. Data from 10 wt cells and 12 ptx1 cells were analyzed.

The hypothesis that there is a second, distinct beating mode can be explored through estimates of the flagellar force $F$ and power $P$ [25]. In a caricature of the power stroke we imagine a straight flagellum of length $L$ pivoting from initial polar angle $\theta_0$ to a final one $\theta_f$ during half the beat period. Using resistive force theory we integrate the viscous force along the filament to obtain

$$F \sim 2 \zeta_L \nu A,$$

where $\zeta_L$ is the perpendicular drag coefficient and $A$ is the waveform area defined previously. A similar calculation yields the power $P \sim (2/3)FV$, where $V = L\dot{\theta}$ is the flagellum tip speed. Ratios of the product $\nu A$ thus serve as measures of relative force in different beats. Restricting to a subset of cells whose flagella were most planar, averaged values of the pairs...
close to the diagonals, but the mean displays remarkably
precise IP and AP motion, with phase coherence main-
tained during power and recovery strokes. Transitions
to and from these two types of synchrony (Figs. 3c,d)
are always initiated by one flagellum, either cis or trans,
which undergoes alteration of beating mode first [13].
Using Poincaré sections we examine the re-emergence of
synchrony during transitions between the modes using
the difference \( (\psi_{\text{lead}} - \psi_{\text{follow}}) / 2\pi \) between the phase
of the flagellum that leads the transition and that which
follows. On a phenomenological level AP→IP and IP→AP
transitions should obey a noisy Adler equation [5]:

\[
\dot{\Delta} = -V'(\Delta) + \xi(t) .
\]  

Here \( V(\Delta) = -\delta \nu \Delta + U(\Delta), \) with \( \delta \nu \) an intrinsic
frequency difference and \( U \) an effective periodic potential
in \( \Delta, \) and \( \xi(t) \) is a noise term. Applying this to ei-
ther type of synchrony in \( ptx1 \) we expect \( \delta \nu \approx 0 \) due
to the lack of flagellar dominance [15]. The most par-
simonious model would then be \( U = -\epsilon \cos(2\pi \Delta) \),
with \( \epsilon > 0 \) for AP→IP and \( \epsilon < 0 \) for IP→AP. Solving for
the deterministic dynamics \( (\xi = 0) \) in a scaled time
\( s = \nu(t - t_i) \) centered at the inflection point of the tran-
sition \( t_i, \) where \( \nu \) is the average IP frequency, we obtain
\( \Delta = -1/(2\pi) \cos^{-1} \tanh(s/\tau), \) with rescaled relaxation
time \( \tau = 1/(4\pi^2 \epsilon/\nu). \) Fits to the data yield \( \tau_{\text{AP→IP}} =
1.65 \pm 0.02 \) and \( \tau_{\text{IP→AP}} = -2.07 \pm 0.04 \) (Fig. 3c,d) and
thus \( \epsilon_{\text{AP→IP}}/\nu \approx 0.015 \) and \( \epsilon_{\text{IP→AP}}/\nu \approx -0.012, \) consist-
ent with the \( wt [5] \).

The necessity to invoke couplings of opposite sign to
account for the AP and IP states within the simplest
model (1) provides a natural starting point for a discus-
sion of mechanisms proposed for synchronization. Two
key issues arise: the structure of the potential \( U \) and
the origin of the coupling constants. With \( \delta \nu = 0, \) the
solution to the Fokker-Planck equation for the probabili-
ity distribution function \( P(\Delta) \) associated with (1) gives
\( \beta U = -\log[P(\Delta)] \) with \( \beta \) related to the noise in
the usual manner. The function \( \beta U \) so determined [26] will
be a bistable potential with local minima at integers and
half-integers. This could be accommodated by higher-
order Fourier components, as \( U(\Delta) \approx -\epsilon \cos(2\pi \Delta) -
\alpha \cos(4\pi \Delta), \) with \( \epsilon > 0 \) and \( \alpha > \epsilon/4. \) An alternative
to this picture of a fixed potential landscape \( U(\Delta) \) with
stochastic hopping between local minima is a fluctuating
landscape switching between potentials \( U_{\text{IP}} \) and \( U_{\text{AP}}, \)
the former with minima only at integers, the latter at half-
integers. Within the limitations of a phase-oscillator de-
scription, the distinction between these views is funda-
mentally a matter of which degrees of freedom are consid-
ered part of the dynamical system and the relative time
scales for those variables. In fact, precedent for a fluctuat-
ing landscape can even be seen in the \( wt [5], \) in which
asynchronous beating (“drifts”) corresponds to a wash-
board potential tilted by a large \( \delta \nu \) so there are no local
minima, while synchronous beating has \( \delta \nu \) small enough
to allow local minima.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{synchronization_dynamics}
\caption{(color online). Synchronization dynamics. Phase
plane of polar angles \( \theta_{\text{cis,trans}} \) reveals the IP (green) and AP
(red) synchronization of a single cell (a), and (b) the average
over 6 cells, averaged over \( O(10^3) \) beats and resampled at 15
points, equally-spaced in time. Shaded regions in (a) indicate
one standard deviation of fluctuations. (c),(d) are sample
timeseries for evolution of \( \theta_{\text{cis,trans}} \) during a transition event.
(e) Phase difference dynamics during AP→IP (orange) and
IP→AP (blue) transitions for 60 events, with means (solid
lines) and standard deviations (shaded), vertically aligned by
plotting difference modulo 1. Dashed lines are fits to data.}
\end{figure}
Models of synchronization based on hydrodynamic coupling often represent flagella by microspheres driven by an internal force. That force may be constant along a trajectory with elastic compliance [9], or the trajectories are rigid and the forcing varies with phase [8]. The mechanism of synchronization in the first class is illustrated in Fig. 4a,b. Measuring the phases (φ₁, φ₂) as indicated, cilia are modelled co-rotating orbits, say ω₁ ≡ φ₁ > 0 and ω₂ ≡ φ₂ > 0. If sphere 1 lags 2 then the flow produced by 1 will push 2 to a larger radius. If the internal force is constant, ω₂ will decrease, and 1 catches up. Conversely, if 1 leads 2 then it pushes 2 inward, so 2 acquires a higher phase velocity and catches up. The flow induced at 1 by 2 leads to consistent results, showing that co-rotating IP motion is stable. To model Chlamydomonas the spheres must be counter-rotating, with say ω₁ > 0 and ω₂ < 0. Then, these considerations, together with anisotropy of the stokeslets, predict stable AP synchronization. Indeed, the coupling constant in (1) scales as ε ∝ −ω₁ω₂ and is negative (positive) for co- (counter-) rotation. In this simple model the AP beating of ptx1 is the ‘normal’ behavior, and the IP mode is anomalous! The situation is not so clear, for if the relationship between radius and phase velocity is reversed then the coupling changes sign [16, 17]. This relationship could be influenced by mechanosensitive cues [27]. In the class of models with forcing that varies with phase angle, synchronization can be understood by similar means in terms of the flow induced by one sphere at the other. Allowing for non-circular trajectories as well as proximity to a no-slip surface leads to the possibility of an effective potential with the higher-harmonic structure discussed above, stabilizing both IP and AP patterns [8, 19]. The difficulty in determining the relevance of these arguments to ptx1 is that the two modes of synchronization are associated with distinct waveforms, with potentially different compliances, internal forcing, and proximity to the cell surface. A third model [18] builds on the fact that transient deviations from locked phases lead to yawing motion of the cell which can produce differential forces on the flagella, bringing them back into phase. While such a mechanism may pertain to free-swimming cells, it is not immediately clear how it can encompass the appearance of both IP and AP states of cells held strongly on micropipettes, where we observe only minute angular displacements (below 1° in both states). The presence of the cell-body itself appears not to be essential for synchrony of the two flagella, for a wt-like breaststroke has been observed in isolated flagellar apparatus (axonemes still connected through their basal bodies), after reactivation by ATP [28].

No existing models of eukaryotic flagella explain the antiphase waveform. Approaches based on optimizing swimming efficiency or nutrient uptake in a model of Chlamydomonas [29] do find a mode comparable to the IP state. Perhaps the AP waveform is not optimal in any conventional sense, but instead exists as one of a discrete number of modes that can emerge from sliding filament models [20]. It will be important to establish whether the higher frequency and distinct waveform are properties intrinsic to a single flagellum, or derive from interactions between the two; key insight may be gained from examining dynamics of uniflagellated double mutants of ptx1.

The physiology of stochastic transitions in the pattern of flagellar beating is currently unknown; we hypothesize that fluctuations in the concentration of a small molecule or ion might be the origin. One candidate would be Ca²⁺, which is isolated and reactivated flagellar axonemes is known to control the waveform [30]. Interestingly, calcium ions are also responsible for the contractility of striated fibers that connect the basal bodies of flagella [31], which in turn may act as a spring with variable stiffness. The current state of this potential spring may influence the preferred mode of synchronization. Indeed, generalizing the orbiting-sphere model [9] to include an elastic connection between flagella bases can lead to stabilization of either IP or AP modes (Fig. 4c), depending on microscopic details. In the simplest linear spring, for example, the AP mode (termed ‘parallel’ by Rüffer and Nultsch [12]) can be selected, for it is the mode in which the relative displacements of the flagellar connections within the cell body are most nearly constant. The role of these fibers for flagellar synchronization may be clarified by altering their mechanical properties by chemical or other means.

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[24] 5 mM HEPES, 40 mM KCl, 10 mM CaCl2, pH 7.2.