gradients, starting from unexpected experimental observations of stochastic response time of individual neutrophils after sudden exposure to spatial chemoattractant gradients. We propose that neutrophils orientation is achieved by the synergy between localized temporal sensing through expanding pseudopods and whole-cell integration of the temporal information by microtubules. In our model, microtubules play functional roles in the local positive feedback via stabilization near membranes experiencing localized temporal concentration increases, and provide global signal integration via scarcity and redistribution inside cells. Experiments using chemical inhibitors of microtubules support the hypothesis that microtubules could play a key role in cell orientation in the presence of spatial chemoattractant gradients. Modeled cells can not only detect the direction of a spatial gradient, but at the same time remain responsive to further changes in the direction of the gradient. Better understanding of neutrophil activity could have practical implications in clinical conditions of inflammation and during immune responses against bacteria and injuries.

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How Does The Bacterial Flagellar Motor Of Rhodobacter Sphaeroides Stop - Using A Clutch Or A Brake?

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The bacterial flagellar motor is a rotary molecular machine ~50 nm in diameter enabling some bacterial species to swim. It is embedded in the cell envelope and connected to an extracellular helical propeller. The motor is powered by the flow of ions down an electrochemical gradient across the cytoplasmic membrane into the cell. Most of our knowledge on motor function comes from work on the *E. coli* motor, which can switch between clockwise and counterclockwise rotation, allowing the bacterial cell to change direction in response to different stimuli.

A proton-driven flagellar motor of *Rhodobacter sphaeroides* achieves the same goal as the bi-directional *E. coli* motor, that of changing cell direction in response to the external environment, but does so by stopping and rotating in only one direction.

We employed several techniques to monitor and manipulate the motor to find out how the stop is achieved. The rotation of a 0.83μm polystyrene bead attached to a truncated flagellum was monitored using back-focal-plane laser interferometry. This allowed us to observe stops in motor rotation with a high temporal (up to 0.1 ms) and angular (~1 degree) resolution. In separate experiments we tethered cells down to glass coverslips by their flagella and applied external torque with an optical trap using the cell body as a handle.

Here we characterize mechanical properties of the motor and show how the motor stops rotating - by putting the brakes on.

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Experimental Evidence for Conformational Spread in the Bacterial Switch Complex

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The allosteric regulation of proteins has classically been understood in terms of the Monod-Wyman-Changeux (MWC) or Koshland-Nemethy-Filmer (KNF) models. These are recognized as limiting cases of a general allosteric scheme that has recently been described in a model of conformational spread. A candidate proposed for testing the model is the bacterial switch complex, an ultrasensitive multimeric protein ring responsible for controlling the direction of rotation of the bacterial flagellar motor. The complex is too large for MWC-type interactions to be applicable and cooperative binding studies have ruled out the KNF model. Here we use high-resolution back-focal-plane interferometry to resolve intermediate states of the complex predicted by conformational spread, and demonstrate detailed quantitative agreement between our measurements and simulations. Individual switch events are not instantaneous, but follow a broad distribution of switch times with mean ~ 20 ms, incomplete switches occur at a bias-dependent frequency and intervals between switches are exponentially distributed at all values of bias.

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Direct Observation Of [Ca2+]i Changes In Motile Sperms With 50 msec Time Resolution

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Ejaculated motile sperms swim against flow in oviduct toward egg. For fertilization, acrosome reaction and regulation of sperm motility including hyperactivation and control of flagellar beat are important events. $[Ca^{2+}]_i$ plays a major role in all the important sperm functions that occur after ejaculation. Much work on sperm Ca^{2+} signaling has used agonists and activators rather than flow, because the small size of sperm presents inherent difficulties in direct observation of motile sperms. Indeed, the $[Ca^{2+}]_i$ in motile sperm has not been directly recorded in flow in microfluidic environment. We will report the system to record $[Ca^{2+}]_i$ in motile sperm with and without flow, and investigated the correlation between velocity of motile sperm and $[Ca^{2+}]_i$ distribution in sperm. Sperm motions in microfluidic environment and $[Ca^{2+}]_i$ changes in the motile sperms were investigated by high-time resolution confocal fluorescent microscopy with high magnification. To record $[Ca^{2+}]_i$, human sperm suspensions stained with FLUO-3AM were injected into a microfluidic channel fabricated by soft-lithography, and confocal fluorescent 4D images were reconstructed with time resolution of 50 msec/frame. $[Ca^{2+}]_i$ changes in the head, midpiece, and tail of the sperm were observed. We found a positive correlation between motile sperm velocity and maximum fluorescent intensity, corresponding to $[Ca^{2+}]_i$, in the midpiece of a sperm. Based on the studies on sperm chemotaxis, $[Ca^{2+}]_i$ is accumulated in the midpiece of sperm, and the Ca^{2+} ions in the midpieces are used for the regulation of flagellar beat mode. We can suggest that $[Ca^{2+}]_i$ elevation in the midpiece would be necessary for high-speed movement of the flagellar.

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Temperature-dependence Of Torque Generation Of The Na ± driven Chimeric Flagellar Motor And Visualization Of The Stator Proteins In E.coli

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Bacterial flagellum is a supramolecular complex and consists of a basal body, a helical filament, and a hook. A basal body embedded in cell membrane functions as a rotary motor driven by electrochemical potential of specific ion, and rotates flagellar filament like as screw. The rotor consists of MS-ring (FliF) and C-ring (FliG, FliM, and FliN).

To learn roles of the electrostatic interaction between stator and rotor in the mechanism of torque generation, we examined the motor response over the temperature range 5-50 degree. At low temperature (23-5 degree), rotational speeds linearly decreased with decreasing temperature. With increasing temperature, however, sudden drops of speeds were observed over ~30, ~40 and ~50 degree. When the temperature returned back to 23 degreee, the speed was restored mostly in several minutes. The drop and recovery of the speed were coincided with stepwise change in the generated torque.

And, we constructed fusion proteins of rotor components and Green Fluorescent Proteins, and investigated whether rotor components are exchanged in a functional motor by FRAP analysis for a single motor labeled with GFP.

In the tethered cell that was produced each GFP fusion, a fluorescent spot was localized at the rotational center. Each GFP fusion was probably incorporated into flagellar motor as a rotor component. In order to investigate the exchange of rotor components, we carried out FRAP analysis using evanescent light. GFP-FliN or FliM-GFP recovery of fluorescence at the rotational center was observed as time passed. On the other hand, the recovery of fluorescence was not observed in the cell producing GFP-FliG. These results suggest that some rotor components assemble to motor even after functional motor is constructed.

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Distribution Of Traction Forces Associated With Shape Changes In Migrating Amoeboid Cells

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Amoeboid motility results from the cyclic repetition of a repertoire of shape changes leading to periodic oscillations of cell area (motility cycle). This study aimed to identify the dominant shape changes and their association to the regulated activity and localization of molecular motors. For this purpose, we applied Principal Component Analysis (PCA) to time-lapse measurements of cell shape, traction forces and fluorescence from the F-actin-binding protein limEΔcoil-GFP in migrating *Dictyostelium* cells. This method provides the most significant cell shape changes of the motility cycle, together with maps of the traction forces and F-actin distribution associated with each shape change mode. It also sorts these modes according to their contribution to the variance of the cell area oscillations observed during the motility cycle. Using wild-type cells (*wt*) as reference, we investigated myosin II activity by studying myosin II null cells (*mhcA-*) and essential light chain null cells (*mlcE-*). The results revealed that *wt, mlcE-* and *mhcA-* cells implement similar shape changes during their motility cycle, although they are implemented at a slower pace in myosin mutants. The repertoire of shape changes is surprisingly reduced as only three modes are