the experimentally observed spindle orientation can be understood as the result of the action of cortical force generators acting on the spindle microtubules. We assume that the local activity of force generators is controlled by the spatial distribution of cell adhesion sites determined by the particular geometry of the adhesive substrate. We develop a simple physical description of the spindle mechanics, which allows us to calculate the torque acting on the spindle, as well as the energy profile and the angular distribution of spindle orientation. Our model accounts for the preferred spindle orientation, as well as the full shape of the angular distributions of spindle orientation observed in a large variety of pattern geometries. Remarkably, it also describes the transition from symmetric to asymmetric spindle orientation, observed for certain changes of the shape of the adhesive patterns. We conclude that, on the basis of a few simple assumptions, we can provide a quantitative description of the spindle orientation of adherent cells. M. Théry, A. Jiménez-Dalmaroni, et al., Nature 447, 493 (2007).

1009-Plat

Taking Control of the Bacterial Flagellar Motor

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The bacterial flagellar motor is a fairly complex machine, requiring 40-50 genes for its expression, assembly and control. Furthermore, it is imbedded in the multiple layers of the bacterial membrane. That explains why, unlike many other molecular motors, it has not been studied in vitro. As spectacular studies of linear motors (like kinesin, myosin and dynein) have clearly demonstrated, an in vitro system provides the essential control over experimental parameters to achieve the precise study of the motor's physical and chemical characteristics. Here, we report significant progress towards the development of a unique in vitro system to study quantitatively the bacterial flagellar motor.

Our system consists of a filamentous Escherichia coli bacterium partly introduced inside a micropipette. Femtosecond laser pulses (60 fs and ~ 15 nJ/pulse) are then tightly-focused on the part of the bacterium that is located inside the micropipette. This vaporizes a submicrometer-sized hole in the wall of the bacterium, thereby granting us access to the inside of the cell and the control over the proton-motive force (pmf). Using a patch-clamp amplifier, we applied an external voltage between the inside and the outside of the micropipette. If the hole in the bacterium is open, that voltage should translate into a membrane potential powering the motors outside of the micropipette. As we varied the applied potential, variations in the motor's rotation speed were observed. For these preliminary results, the rotation speed was observed directly using video microscopy of fluorescently labeled filaments. That system opens numerous possibilities to study the flagellar motor and other membrane components.

1010-Plat

How Molecular Motors Shape The Flagellar Beat

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Cilia and eukaryotic flagella are slender cellular appendages whose regular beating propels cells and microorganisms through aqueous media. The beat is an oscillating pattern of propagating bends generated by dynein motor proteins. A key open question is how the activity of the motors is coordinated in space and time. To elucidate the nature of this coordination we inferred the mechanical properties of the motors by analyzing the shape of beating sperm: Steadily beating bull sperm were imaged and their shapes were measured with high precision using a Fourier averaging technique. Comparing our experimental data with wave forms calculated for different scenarios of motor coordination we found that only the scenario of interdoublet sliding regulating motor activity gives rise to satisfactory fits. We propose that the microscopic origin of such ''sliding control'' is the load dependent detachment rate of motors. Agreement between observed and calculated wave forms was obtained only if significant sliding between microtubules occurred at the base. This suggests a novel mechanism by which changes in basal compliance could reverse the direction of beat propagation. We conclude that the flagellar beat patterns are determined by an interplay of the basal properties of the axoneme and the mechanical feedback of dynein motors.

1011-Plat

Mechanics Of Neutrophil Motility On Compliant Gels Measured With Traction Force Microscopy

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Traction force microscopy (TFM) allows imaging of the traction field exerted by a cell during adhesion and spreading on an elastic hydrogel. We used a combination of TFM and microfluidics to measure the traction forces and motility of human neutrophils under both chemokinesis and chemotaxis in response to formyl-met-leu-phe (fmlp). Using polyacyrlamide gels functionalized with intercellular adhesion molecule-1 (ICAM-1), we show that neutrophil traction stresses can be measured across a broad range of gel stiffnesses, from 6 to 20 kPa. We found neutrophil directed motion is caused by a rearward squeezing uropodial stress, and the cell motion is always counter to this motion; this is true both in chemokinesis as well as chemotaxis. During turning, the orientation of the rearward stress precedes turning. Cells exert larger forces in chemotaxis (r.m.s. force of ~ 100pN) than in chemokinesis (~ 50 pN). On surfaces of different compliance, cells move with a greater force and a higher chemotatic index on stiffer substrates; these changes occur without a change in neutrophil speed. In the same magnitude gradient, cells move directedly and with greater force if the mean concentration of chemoattractant is closer to the K_D of receptor binding. Blocking with an antibody against β_2-integrin (TS1/18) completely eliminates traction forces and directed motion. RhoA has been implicated as signal transduction agent in the cell that is responsible for rearward contractile stress and the direction of neutrophil motion; we show here that inhibition of a GTPase down stream of RhoA (ROCK) with a pharmacological agent reduces directional motion and force generation, and leads to abnormal morphology in which rearward contraction is compromised. Taken together, neutrophil directed motion and force generation result from an interplay between substrate adhesiveness and uropodial contractility through RhoA.

1012-Plat

Mechanics in neuronal development

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Nervous tissue consists of several different types of cells, blood vessels, and extracellular matrix. All these building blocks differ in their mechanical properties. Particularly during growth and migration, the local mechanical environment of neurons may thus change dramatically. The softness of radial glial cells, along which neurons preferentially grow, and the neuronal preference for soft substrates strongly point towards a role of mechanics in neuronal guidance. Here we show how neurons detect and avoid stiff substrates and how their mechanoresponsiveness is used to guide their axons along distinct pathways.

In vitro, neurons continuously probe the mechanical properties of their environment. Growth cones visibly deformed substrates with a stiffness commensurate with their own compliance. To understand the growth cones' sensing of stiff substrates, we investigated their precise temporal response to well-defined mechanical stress. Externally applied stress exceeding the threshold of ~300 pN/μm^2 caused a calcium influx through mechanosensitive, stretch-activated ion channels in the growth cone membrane that triggered neurite retraction. Subsequently, neuronal processes re-extend, thereby enabling exploration into new directions.

When *Xenopus* eye primordia were cultured on polyacrylamide gels of different compliance, the morphology of the outgrowing retinal ganglion cell axons dramatically depended on the mechanical properties of their substrate. If the axons grew either on soft or on stiff surfaces, they spread over a wide area to explore different directions. In contrast, if they grew on substrates of intermediate compliance, they fasciculated and grew into one common direction, resembling an optic nerve. The concerted growth along pioneering axons depended not only on the substrates' compliance but also on that of the axons themselves. Hence, neurons may actively use mechanics as previously unknown guidance cue during growth and migration. This knowledge may ultimately help in finding new implants that promote axonal regeneration in the injured nervous system.