produced by the *motB(D33E)* mutant motor was about half of the wild type while it was recovered nearly to the wild-type levels for the pseudorevertants. However, their high-speed rotation under low load was still significantly impaired.

These results together suggested that MotB(D33E) mutation reduced both proton-conducting activity and torque generation involving the stator-rotor interactions coupled with protonation/deprotonation of Glu-33. Furthermore, the second-site mutations could recover the torque generation but not the proton-conducting activity.

Recently, to measure the proton-conducting activity of the *motB(D33E)* mutant and its pseudorevertants, we developed a novel system to monitor intracellular pH of cells overexpressing MotA/MotB mutant proteins utilizing pH-sensitive GFP (pHluorin). Details of these results will also be discussed.

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Probing the Bacterial Flagellar Motor using Temperature Control

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Accurate temperature control is useful to probe different energetic and kinetic regimes of any molecular motor. Here we use a peltier-cooled objective to examine torque speed curves and single stator speeds of the bacterial flagellar motor in E. coli between 5C and 22C. We observe that in the high load regime, the temperature effects are minimal, whereas in the low load regime the reduction in speed due to cooling is significant. In addition we measure the membrane voltage across our temperature range to verify that the ion-motive-force does not change with temperature, as predicted from the low-load temperature independence.

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Dynamic Viscoelasticity Of Individual Bacterial Cells

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Understanding how the shape of cells is regulated in time and space during cell growth and division is a fundamental problem of contemporary biology and requires quantitative estimates of cell mechanical properties. In the present paper, we have used an AFM-based approach to probe the mechanical properties of single bacterial cells (gram-negative *Escherichia coli* K12) by applying a constant compressive force to the cell under fluid conditions while measuring the time-dependent displacement (creep) of a colloidal AFM tip due to the viscoelastic properties of the cell. It was seen that the cells exhibited a viscoelastic solid-like behavior with retarded elasticity, i.e. both an instantaneous and a delayed elastic deformation. We found that this behavior is well described by a three-parameter mechanical model. Using the best fit parameter values, we have calculated the dynamic viscoelastic behavior of the cells over a wide range of frequencies based on a numerical time-frequency transform technique and we have compared the calculated behavior with that measured experimentally. Comparison of the results obtained for *E. coli* with previously reported data on the mechanical properties of others gram-negative bacterial cells and their isolated surface layers suggests that the elastic component of the cell viscoelastic response is dominated by the properties of the peptidoglycan layer, whereas the viscous component likely arises from the liquid-like character of the cell membranes. This work represents a new attempt to understand how molecular structure leads to the cell mechanical properties, which could be of practical value for elucidating the biomechanical effects of drugs on pathogenic bacteria.

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Bundle-forming pili from enteropathogenic Escherichia Coli generate moderate forces

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Enteropathogenic Esherichia coli (EPEC) is a causative agent of diarrhea in humans, rabbits, dogs, cats and horses and a leading cause of infant mortality in developing countries . The bundle forming pili (BFP) is a filamentous appendage at the bacterial surface, which is of prime importance in EPEC infectivity. BFP mediates attachment to host cells and formation of bacterium microcolonies. This type of filaments is also involved in motility, biofilm formation, and horizontal gene transfer. No measurement of EPEC BFP ability to exert force has been described to date. Using an array of force sensors made of elastic pillars we observed and quantified the retraction forces exerted by BFP ($35{,}08 \pm 8.94$ pN). We observed that the force was able to build up over time to a maximum force in the order of 80 pN and to hold several seconds up to a minute. We demonstrated that BFP applied forces both on the substrate and on neighborhoods bacteria. Those results showed the existence of a force generating mechanisms in EPEC adhesion and microcolonies formation, two events required for a full infectivity.

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Improved Specimen Preparations for Electron Microscopy of FtsZ Protofilaments

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FtsZ is a bacterial tubulin homolog that can polymerize into protofilaments and other structures in vitro. These in vitro structures have mostly been visualized by negative stain electron microscopy, but poor staining is a persistent problem. We have tested a previously described but underutilized technique of UVO (UV-Ozone) treatment of carbon coated grids (Burgess et al., J Struct Biol 2004). We mounted a low-pressure, mercury-vapor discharge lamp (Spectroline, Westbury,NY - short wave UV quartz pencil lamp, model 11SC-1) 5 cm from the grids and exposed them to UVO for 30-60 min (the lamp and grids are covered with a cardboard box). Negatively stained samples on UVO grids gave at least equal and usually better results than those treated by glow-discharge. Additionally, this method does not require a separate instrument, only a small pen-lamp and a box; thus it is also a cheaper and space-saving option. An alternative to negative stain is rotary shadowing, but the high glycerol concentrations required normally inhibit FtsZ polymerization. We have overcome this problem by using uranyl acetate (UrAc) as a fixative (Zhao&Craig, J Struct Biol 2003) prior to the rotary shadowing procedure, and have obtained images of intact FtsZ filaments. A drop of FtsZ filaments in assembly buffer was spread onto a small square of mica either by spraying or mica sandwich. The side of the square with filaments bound was then touched sequentially to drops of 2% UrAc (fix) and 0.2M ammonium acetate/30% glycerol (wash/shadowing buffer), drained and mounted on the vacuum evaporator stage for shadowing. The main advantage of rotary shadowing is high contrast, low background and high reproducibility of sample ''staining''.

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Morphology of C. Crescentus and Crescentin

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One of the important questions in bacteriology is why different species of bacteria have different morphology. In order to answer this question, first one should know the underlying mechanism for the shape maintenance. Yet this underlying mechanism for determining different shapes is not well understood. As a first step toward understanding bacterial morphology, we investigate the morphology of Caulobacter crescentus (C. crescentus) which is an asymmetric gram-negative bacterium forming a helical crescent-shaped filament. It is experimentally known that crescentin, intermediate filament (IF) homolog of C. crescentus, is required for maintaining this asymmetric bent shape. In this study, we assume that crescentin is a bundle of coiled-coils, since it is a homolog of eukaryotic IF such as keratin, etc. Treating bacterial cell wall and the filament as continuum bodies, elastic energy of the cell wall and the filament attached to the inner side of the surface is computed. By considering energy-minimum shapes obtained by the optimization technique, the elastic and the geometric properties of crescentin in order to maintain the helical shape of C. crescentus is derived. We also investigate the characteristics of the binding type between the cell wall and the corresponding filament. Finally, the dynamic effect of cell wall together with the crescentin on the shape maintenance is speculated.

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MreB, A Prokaryotic Actin Homologue, Contributes To Cell Stiffness In E. Coli

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Genetic and microscopy studies have shown that the newly discovered bacterial cytoskeleton is essential for the maintenance of cell shape in many prokaryotes. Moreover, the localization of different components of the cytoskeleton correlates with the localization of cell-wall-synthesis enzymes and of newly added cell-wall material. These correlations suggest that the cytoskeleton controls cell shape by regulating the location of cell-wall synthesis. However, whether the cytoskeleton also applies mechanical forces inside of cells is unknown. To address this issue, a quantitative measurement of the role of cytoskeletal proteins in cell mechanics is needed.

We used an optical trap to measure the elasticity of live E. coli cells. Upon the addition of A22, a drug that promotes the disassembly of MreB filaments, cells become significantly weaker in their resistance to cell bending. This effect is reversible, and cells recover wild-type stiffness when A22 is removed from the environment. Our results show that MreB, a bacterial actin homologue, contributes as much to the stiffness of a cell as the peptidoglycan cell wall. This is the first direct evidence that the bacterial cytoskeleton contributes to the mechanical integrity of a cell in much the same way as it does in eukaryotes.