B. Tuesday, July 25

Session 1: Molecular Perspectives

Julio Fernandez (Mayo Clinic) discussed the mechanical stretching in vivo, which is thought to regulate the function of many proteins. The application of mechanical force to biological polymers produces conformations that are different than those that have been investigated by chemical or thermal denaturation, and are inaccessible to conventional methods of measurement such as NMR spectroscopy and X-ray crystallography. Force-induced conformational transitions may therefore be physiologically relevant, and may offer novel perspectives on the structure of biomolecules. Recent developments in single molecule force spectroscopy have enabled study of the mechanical properties of single biological polymers. For example, the force-measuring mode of the atomic force microscope (AFM) is capable of measuring force-induced domain unfolding in proteins. Furthermore, through the use of protein engineering, we have examined the mechanical stability and topology of immunoglobulin and fibronectin protein modules which are common muscle and cell adhesion proteins. These experiments have demonstrated a number of mechanical phenotypes that are readily captured by the single molecule AFM technique. We recently demonstrated that point mutations can have large effects on the mechanical stability of an immunoglobulin module. Hence, the AFM may help to elucidate the molecular determinants of mechanical stability in proteins and the role of force-induced conformational changes in the regulation of their physiological function.

Klaus Schulten (University of Illinois, Urbana-Champaign) discussed the structure, dynamics, and function of biopolymer aggregates, including lipids and water forming membrane bilayers, proteins complexing with DNA and regulating gene expression, and proteins involved in complexes with other proteins. Schulten uses very-large-scale computer simulations to study their behavior.

John Frangos (University of California, San Diego) discussed fluid shear stress (FSS) which has been shown to be an ubiquitous stimulator of mammalian cell metabolism. While many of the biochemical transduction pathways have been characterized, the primary mechanoreceptor for FSS remains unknown. His hypothesis is that the cytoplasmic membrane acts as the receptor for FSS. He proposes that FSS increases membrane fluidity, a change that leads to the activation of heterotrimetric G proteins (Gudi et al, PNAS 90: 2515-2519, 1998). 9-(dicyanovinyl)-julolidine (DCVJ) is a fluorescent probe that integrates into the cell membrane and changes quantum yield with the viscosity of the environment. In a parallel-plate flow

chamber, a confluent layer of DCVJ-labeled human umbilical cord venous endothelial cells were exposed to different levels of FSS. With increased FSS, a reduced fluorescence intensity was observed, indicating an increase of membrane fluidity. Step changes of FSS caused an approximately linear drop of fluorescence within 5 seconds, showing fast and almost full recovery after shear stopped. A linear relationship between shear stress and membrane fluidity changes was observed. This study clearly shows the direct link between fluid shear stress and membrane fluidity, and suggests that the membrane may be the primary flow mechanosensor of the cell.

Session 2: Cellular Perspectives

Gabor Forgacs (University of Missouri) discussed a general network model for information transmission by diffusion along cytoskeletal elements. This model was contrasted to the current simple diffusional models for soluble signals. He outlined a method of magnetic bead rheology with which he hopes to test the model, although some listeners were unclear about what specific rheological predictions the model makes other than some evidence of network structure. He also introduced a novel magnetic tweezer apparatus, capable of producing forces of orders of magnitude stronger than existing tweezers. He is planning to use this apparatus to investigate the proposed interconnected nature of the cytoskeleton. In connection with his talk Michael **Sheetz** reminded that he had earlier demonstrated the possibility for microtubule associated proteins to indeed diffuse along these filaments, thus giving support to the suggested mechanism of signaling. Alan **Hunt** noted that there must exist a lower size cutoff for molecules diffusing along cytoskeletal filaments. Below this cutoff he expects free diffusion to be the principal mechanism for intracellular protein translocation.

Steven Heidemann (Michigan State University) argued that the tensegrity model of intracellular architecture is too specific to explain a number of observations. In particular he argued that “tensegrity lacks time scale aspects”, cortical tension is not the primary determinant of cell shape and stress hardening (being an important feature of tensegrity) characterizes also the cell models of Hiramoto (rubber model) an of Yonegida (liquid drop model). He cited Fuller’s statement that tensegrity in no way mimics living structures. He described experiments in which GFP labeled cytoskeletal proteins had been used to follow the consequences of pulling on cytoplasmic processes. Since the applied forces produced only local responses, he concluded that the results of these experiments, performed on fibroblasts, are inconsistent with the predictions of the tensegrity model. He noted that tensegrity still may be a useful representation for other cell types (i. e. neurons).

Donald Ingber (Harvard University) defended the tensegrity model. He disputed the arguments of Heidemann and reasoned that tensegrity is the only structure which has built in prestress necessary to understand a number of cellular phenomena. He presented experimental results in favor of the model. In particular, he has shown that disrupting the actin cytoskeleton leads to the same effect as changing cell shape (which he and his collaborators can do in a controlled manner using special "moulds"). He argued, this finding is consistent with the tensegrity model. Furthermore, he showed that when the cell spreads, so does its nucleus, which (according to him) can be understood only if a prestressed tensegrity structure extends in the interior of the cell including the nucleus.

Discussion Summary:

Alan **Hunt** asked whether the tensegrity model can be used to understand structure from the atomic scale all the way to cosmic scales, to which **Ingber** responded that indeed it can. Christian **Oddou** noted that numerous experimental results obtained in his lab, using stick and string representation of cytoskeletal filaments are consistent with the predictions of the tensegrity model and as long the model does not fail, it should not be abandoned. Several participants stressed that tensegrity structures as conceived by Buckminster Fuller are passive engineering constructions and they are not necessarily correct representations of the rapidly varying cytoskeleton, with these variations being controlled by gene activity.

These talks and following discussions indicated a consensus on the role of the cytoskeleton in intracellular force transduction. Although a number of experimental observations can be explained by assuming the cytoskeleton to be an interconnected network of specific filaments (either via a percolation or a tensegrity structure), other observations seem to inconsistent with this hypothesis (at least with the model based on tensegrity). Thus, the topic remains contentious and further studies are needed to clarify the precise mechanism through which the cytoskeleton may participate in intracellular signal and force transmission.

Session 3: Tissue/Organ Perspective

The topics in this session included asthma, muscle implants and hearing, all three are relevant to human health. The speakers presented tissue/organ perspectives based on molecular and cellular mechanisms.

Roger D. Kamm (Massachusetts Institute of Technology) began the session by describing asthmatic tissue remodeling that decreases the dimensions of the airway. His central hypothesis is that airway remodeling is a response to a mechanical stimulus rather than generalized inflammation. He went on to present results based on *in vitro* culture models

showing the mechanical stimulus (most likely shear stress) is transduced by epithelial cells into a biochemical signal that acts on co-cultured fibroblasts.

Herman H. Vandenburgh (Brown University) followed with a description of bioartifical muscles (BAMs). BAMs are fabricated from mammalian skeletal muscle stem cells. A variety of strategies involving both the intensity and temporal properties (including quiescence) of applied stress were described for guiding the modeling of this tissue. The goal was to enhance its ability of generate mechanical force. BAMs are less efficient than native muscle *vis a vis* force transduction but they have potential for therapeutic protein delivery. Genetic induction of protein expression reveals they are able secrete therapeutic proteins (growth factors, kinases, etc.) at high levels.

William E. Brownell (Baylor Medical School) then described how electromechanical force transduction by outer hair cells enhances mammalian hearing. Outer hair cells provide a positive feedback of mechanical force that counteracts viscous damping forces. The cells convert electrical energy directly to mechanical energy at frequencies >100 kHz. Experimental evidence locates this piezoelectric-like force generator in the plasma membrane of the cell's lateral wall. Electromechanical force transduction has not previously been associated with membranes. The potential for membranes to provide useful work is a novel biological and physical concept.