II. Summary of Invited Talks

A. Monday, July 24

Session 1: Molecular Perspectives

The theme of this session was the application and measurement of forces in biological systems ranging from the single molecule to whole, living cells. Force can be used as a tool to study and even change the structural state of individual biomolecules such as DNA and proteins. At the whole cell level, the forces generated by living cells play a key role in cell motility and shape. Crucial in understanding the generation of force and the resistance of cells to external forces is a characterization of the transmission of force into the cell body from the external environment. Likewise, the characterization of the material properties of the basic constituents of the cell is important.

The recent development and use of single-molecule manipulation techniques, such as various trapping methods, has permitted the study of single biomolecules in ways not possible just a few years ago. **David Ben-Simon** (Ecole Normal Superior, Paris) described some of the recent efforts to study single DNA molecules and associated enzymes. Specifically, force can be used as a tool to both examine and *change* the structural state of single DNA: different states can be induced by a combination of twist and stretch manipulation. It has also become possible recently to study the activity of single enzymes, such as DNA-polymerase and the relaxation of torsion by topoisomerase, which are important in the packing and transcription of DNA.

Michael P. Sheetz (Columbia University) focused generally on methods to measure cell mechanics *in vivo*, and specifically on the role of integrins in force transduction. Cells generate and respond to forces in part via integrin-matrix contacts, which are highly dynamic and which involve enzyme processes. The mechanisms of force generation and response in living cells have been studied using sensors based on silicon chips, together with laser tweezers. The various stages (extension, adhesion, and reinforcement) of cell motility have been characterized in this way.

An understanding of the generation and transmission of force in cells requires a basic understanding of the properties of the complex materials that constitute the living cell, as well as tools for their characterization. The cytoskeleton, which consists of a complex network of filamentous proteins or biopolymers, plays a key role in this force response of eukaryotic cells. Many recent efforts have focused on the characterization of such viscoelastic materials *in vitro*

and *in vivo*. **Frederick M. MacKintosh** (University of Michigan and Vrije University of Amsterdam) summarized some of the principles and recent techniques of such *microrheology*, as applied to soft and biological materials. These techniques have been developed both to characterize bulk materials at a micrometer scale, as well as to probe small samples such as whole cells.

Session 2: Cellular perspectives

Peter F. Davies (Univ. of Pennsylvania) reviewed length scales of hemodynamic forces acting on the endothelium of blood vessels. Shear stresses were shown to regulate vascular endothelium over length scales ranging from tens of centimeters-millimeters throughout the tortuous geometry of the arterial tree and at flow separations, to micron scale at the topographic surface of individual cells within the endothelial monolayer, to sub-microns-nanometers during intracellular force transmission. Examples of hemodynamic-generated force quantitation at these different length scales were linked to the biological responses (including mechanotransduction signaling, gene transcription effects) and pathological consequences of hemodynamics (eg location of atherossclerotic lesions). New studies demonstrating 4-dimensional, near-real time imaging of endothelial GFP-cytoskeleton revealed spatially-defined displacement of filaments in response to external shear stress applied at the upper cell surface.

Eliot L. Elson (Washington University) described traction forces in a mutant Dictyostelium amoeba that lacks myosin II. These organisms locomote at approximately half the speed of the wild-type form (myosin II positive). Common to both types is rearward particle transport during traction but transport patterns are different in the mutant where a (undefined) low capacity alternative motor appears to operate. Myosin II was also shown to be unnecessary for cell spreading; its contractile forces actually resist cell spreading. The contributions of actin were reviewed in this system in the context of the balance between protrusive forces (actin polymerization) and restrictive forces (myosin).

Raymond E. Keller (University of Virginia) illustrated the dynamic cellular changes during Xenopus gastrulation and neurulation, events that occur through narrowing and elongation of the tissue over several hours. Dr Keller introduced biomechanical forces as a new consideration in these fundamental developmental processes that involve massive rearrangement of cellular components. Force and uniaxial compressive stress relaxation measurements revealed dorsal axial and paraxial tissue forces in the range of 0.6 microNewtons and 3-4 fold increases in stiffness in the axis of extension. Interference with the mechanical status of the tissue resulted in inappropriate development. Studies of component cells removed

from the tissue are inadvisable because their behavior is context-defined. A discussion of the connections between gene-directed aspects and the role of biomechanics in these developmental processes concluded that the physical forces play an important role.

Session 3: Organ Perspectives

Alan J. Grodzinsky (Massachusetts Institute of Technology) presented a talk entitled "Chondrocyte Mechanotransduction: Cellular, Intracellular, and Molecular Responses to Tissue Level Forces." Extracellular matrix (ECM) adaptation to biomechanical demands in dense connective tissues such as cartilage is dependent on the ability of cells to sense and respond to physical stimuli. Recent studies suggest that there are multiple regulatory pathways (e.g., upstream signaling, transcription, translation, and post-translational modifications) by which chondrocytes in cartilage respond to mechanical stimuli and thereby alter the quantity and quality of newly synthesized ECM macromolecules. *In vitro* model systems including cartilage explants and 3-D chondrocyte-gel culture systems have been important in the study of mechanisms of mechano-transduction. Investigators have demonstrated that tissue shear and dynamic axial compression can each stimulate increases in proteoglycan and collagen synthesis and deposition in the ECM. Both static and dynamic compression of chondrocytes in intact tissue explants and in alginate gel culture can also alter the expression of aggrecan and type II collagen mRNA. However, mechanically-induced changes in synthesis are not necessarily dependent on gene transcription. Changes in the morphology and packing of intracellular organelles (e.g., rER, Golgi apparatus, nucleus, and mitochondria) induced by static compression may also regulate the processing and structure of molecules such as aggrecan. Finally, mechanical loading associated with joint cartilage injury is also a risk factor for development of OA. Studies *in vitro* have shown that injurious mechanical compression of cartilage can cause an increase in the number of apoptotic cells in a dose dependent manner.

Stephen C. Cowin (City University of New York, City College) presented a talk entitled "A possible resolution of a paradox in bone mechanosensation." Living bones adapt their structure to meet the requirements of their mechanical environment. These adaptations require a cell-based mechanosensing system with a sensor cell that perceives the mechanical deformation of the mineralized matrix in which it resides. One of the most perplexing features of this mechanosensory system in bone is the very low strain levels that a whole bone experiences *in vivo* compared to those needed to produce a cellular response *in vitro.* Strains *in vivo* depend strongly on frequency; they mostly fall in the range 0.04 to 0.3 percent for animal locomotion and seldom exceed 0.1 percent. These strains are nearly two orders of magnitude

less than those needed (1% to 10%) to elicit biochemical responses *in vitro,* such as an increase in intracellular Ca2+ and prostaglandin synthesis. There is a paradox in the bone mechanosensing system in that the strains that activate the bone cells are orders of magnitude larger than the stains to which the whole bone organ is subjected. A hierarchical model, ranging from the subcellular level to the whole organ level, is used to resolve this paradox. Using this model, it is possible to explain how the fluid flow through the pericellular matrix surrounding an osteocytic cell process can lead to strains in its actin cytoskeleton which are two orders of magnitude greater than the mineralized matrix in which it resides.

Janet Braam (Rice University) presented a talk entitled "Molecular and Developmental Responses of Plants to Mechanostimulation." Despite their passive appearance, plants sense and actively respond to environmental stimuli, including mechanical stimuli like touch. Wind blown or touched plants will undergo altered development such that they are more resistant to mechanical stress. In Arabidopsis, there are strong and rapid gene expression responses to touch. These genes, called the TCH genes, encode calmodulin, calmodulin-related proteins and a cell wall modifying enzyme. Investigation of the regulation and functions of the TCH genes is being used to attempt to uncover the mechanisms by which plants sense mechanical force, transduce signals into cells, and modify growth patterns.

Both plant and animal tissues adapt their shape and form to the mechanical loadings to which they are subjected. While this influence is particularly strong when the tissue is growing, it also occurs in mature tissues. The three talks in this session consider a sample of plant and animal tissues that demonstrate this stress adaptation, articular cartilage, bone and several plant tissues.

Contemporary research has as its objective the description of the cellular and molecular mechanisms that make this structural adaptation possible. Generally these mechanisms involve sensor cells, material (protein) manufacturing cells, deconstruction (phagocytic) cells and systems of inter- or intra- cellular communication. The specifics of these features vary between tissue types, but all feature mechanosensation.