Report of the NSF Workshop on Force Transduction in Biology July 24-26, 2000 Arlington, VA

David A. Weitz
Harvard University
and
Paul Janney
University of Pennsylvania
(co-chairs)

This material is based upon work supported by the National Science Foundation under Grant No. 0084451. Any opinions, findings, conclusions, or recommendations expressed in this report are those of the participants, and do not necessarily represent the official views, opinions, or policy of the National Science Foundation.

SUMMARY

Transduction of forces is ubiquitous in biology: Gravity exerts a stress on all objects, cell motion involves forces between cells and their surroundings, chromosomes are actively separated during mitosis and cell division. It is an important fact, often only implicitly recognized, that these forces originate over a wide range of length scales, from single molecules, to individual cells, to tissues, and to complete organs. Thus, scientists from many disciplines such as biology, medicine, chemistry and engineering, carry out research on biological force transduction. However, for technical and disciplinary reasons, specific projects tend to focus on a narrow range of length scales, such as the molecular scale, the cellular scale or the full organ or tissue scale. The impetus for the Workshop was the notion that it would be useful to address the problem of biological forces, representing many length scales, within a single venue. Such a meeting had not previously been held. Over 100 scientists representing a range of disciplines attended the three-day Workshop. The goals of the Workshop were: (1) To present recent advances in research on biological force transduction, (2) To identify new, interdisciplinary or synergistic interactions that could speed progress, and (3) To make recommendations about resources or mechanisms needed to achieve these opportunities.

Each day included a session of three invited talks in each of the three areas represented at the Workshop: molecular, cellular and tissue research. There were also short contributed presentations from 28 of the participants. Two evening panel discussions were held. One dealt with common research problems, synergies and interactions. The second focused on potential federal funding opportunities, and involved representatives from NIH, NASA and NSF. The Workshop Report provides an **Overview** of the Workshop, a **Summary of Presentations**, and **Conclusions** to guide future work in the area of force transduction in biology.

Contents

I. Overview

The transduction of forces in biology plays an essential role in a wide range of phenomena, from cell division to response of tissues to gravity. An unusual feature of these forces is that they often are transmitted over multiple length scales. However, biological force transduction problems are normally discussed within the context of relatively narrow disciplinary meetings, characterized by a particular scale, such as a molecule, cell, etc. The impetus for the Workshop was the realization that, owing to recent developments, important problems of biological force transduction over multiple length scales could be usefully discussed within a single venue. This Workshop aimed to explore recent advances, particularly at the molecular and cellular level, and to make special efforts to bridge the discussion to important problems of interest to workers at the tissue and organ level. Over 100 scientists representing a range of disciplines attended the Workshop. The attendees gathered over a three day schedule, with the following objectives: (1) To present recent advances in research on biological force transduction, (2) To identify new, interdisciplinary or synergistic interactions that could speed progress, and (3) To make recommendations about resources or mechanisms needed to realize these opportunities.

A number of major points emerged during the Workshop discussions:

A. Importance of problems involving Force Transduction:

Force transduction in biology clearly impacts a very wide range of phenomena, ranging from single molecular events to large organ response, and with important effects occurring at all intermediate scales. This essential conclusion became clear from the talks presented and from the wide ranging discussions at the workshop. The study of problems involving force transduction in biology has become sufficiently widespread that there is a now a critical mass of researchers involved in this area. While progress is being made, there is unmet need and opportunity for yet greater progress. Force transduction problems are not just a set of biochemical issues, but span the molecular to the macroscopic, with new tools and concepts needed across the spectrum.

B. Emerging research Themes:

The relationship between force transduction in biology and the biological material itself represents an important stand-alone topic. Thus, the study of force transduction has significant potential for development of new types materials, as the mechanisms for force transduction are

better understand. Indeed the “materials and mechanical” properties of biomolecules are major determinants in their biological function. This is qualitatively different from the traditional view of chemical reactivity as the primary “mode d'emploi” of biomolecules. This represents a new frontier for research, one in which the NSF is ideally poised to make a major contribution.

Force transduction problems require new engineering tools and models at the macro end of the spectrum. Engineering models that accurately represent the complex mechano-structural elements in tissues and organs are currently not available. At the molecular end, the properties and function of mechano-sensitive channels are poorly understood, this includes the relation between the mechanical stress or shear state of a molecule and its functionality. While the mechanical function of the cytoskeleton and related molecules is widely studied, a fundamental or predictive understanding of its biological function remains on the horizon.

A unifying concept, that is somewhat difficult to quantify, is the fact that 'biological force' is typically a cascade of events, some chemical, some physical, some genetic. The “Grand Challenge” is to determine this ‘cascade’ for important examples: Morphological adaptation to stress, chemical reaction to stress and gravitropism. These issues need to be further clarified by experts in the field.

C. Communication between disciplines:

The complexity and multidisciplinary nature of the problems involved in force transduction demand extensive interdisciplinary interaction. A full understanding of the vast majority of the crucial problems will require the participation of chemical, biological and physical sciences. However, there is currently difficulty in communication between disciplines, and, unless this is overcome, it will continue to impede progress in the field. There is a strongly perceived need for cross-training at the post-doc level. It was felt that targeted post-docs in this area of research were an immediate need. These post-docs would encourage and promote interdisciplinary research, and provide leadership for the future in the field. There are unrealized opportunities for collaboration between and theory and experiment. In general, theoretical efforts lag experiment largely due to issues of complexity. Again, this represents a significant arena for new research.

D. Research Training in Force Transduction in Biology:

Other means to encourage interdisciplinary interactions that were discussed include the organization of a Gordon Conference on this topic, a lab course taught at the Marine Biology Lab, and application to the NIH for support of a large scale project focussed on force transduction in biology. Each of these suggestions has both advantages and disadvantages. A Gordon conference has the advantages of being low cost, informal, and stimulating direct contacts, and flexible types of connections. It has the disadvantage of providing little in the way of direction, having a slow timetable, having no funding component, and no direct peer-reviewed involvement. A course at MBL has the advantage of being relatively low in cost, helping to train the next generation, and stimulating meaningful interactions between labs. It has the disadvantage of providing little in the way of direction, having a slow timetable, and being elective and possibly exclusionary. A large NIH project has the advantage of stimulating meaningful interactions between labs around problems, providing a way to direct research to specific problems, and involving peer review to help ensure quality. It has the disadvantage of requiring someone to organize a good group, of possibly excluding young faculty because of tenure concerns, and of being expensive, although the NIH has funded large projects such as this in the past.

E. Participation of Young Scientists

Participants in the workshop included about 15 graduate students and postdocs, all of whom actively engaged in the discussion. Their disciplines were as varied as were those of the more senior participants. This afforded them the opportunity to experience first hand the breadth of the interdisciplinary nature of the research required to make progress in this field. Their participation was one of the most positive aspects of the Workshop.

F. Funding Issues:

There was a sense that many problems of mechano-transduction at the cellular level could be solved in the near future, provided sufficient resources are directed to this field of research. However, new initiatives are required for the simple reason that many topics in the field of biological force transduction fall between current areas of emphasis at the major Federal funding agencies, NSF and NIH. Some topics would benefit from a group approach: Understanding mechanotransduction at the molecular level involves understanding of the physical and biochemical processes involved, and requires a battery of experimental and

theoretical skills. It is therefore critical to develop alliances between researchers who know how to manipulate cells and researchers who know how to make and interpret physical measurements at the cellular level. These points were noted in discussions between conference participants and representatives from NIH, NASA, and NSF. Finally, there was also a consensus that closer coordination and development of joint programs between the NSF and the NIH in the area of biological force transduction would be highly productive.

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.

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.

C. Wednesday July 26

Session 1: Molecular Perspectives

Fred Sachs (State University of New York, Buffalo) presented a talk entitled "A blocker for cationic SACs, from channels to animals." He discussed how a 4 kD peptide isolated from tarantula venom blocks cationic SACs with an affinity of about 500 nM. The peptide noted GsMTx-4 is specific for SACs. It doesn't affect steady state I/V curves of heart cells or of astrocytes. It does, however block stretch induced effects. It reduces volume activated currents in astrocytes and can block atrial fibrillation induced by dilatation in the rabbit heart with affecting the action potential.

Evan Evans (Boston University, University of British Columbia) presented a talk entitled "Exploring the Complex Relation between Force – Time – Chemistry in Single Biomolecular Bonds." He discussed how noncovalent-macromolecular bonds are the fundament of nanoscale chemistry in recognition, adhesion, signaling, activation, regulation, and a host of other processes from outside to inside cells. But not well-appreciated is that energy landscapes of these biomolecular bonds are rugged terrains with more than one prominent activation barrier. Near-equilibrium kinetics in conventional test tube assays only reveal a single-outer barrier, which is the classical paradigm of biological chemistry. However, when bonds are detached under a large range of loading rates (force/time), the measurements of single bond strength on a scale of Log(loading rate) provide a spectroscopic image of prominent energy barriers traversed along the force-driven reaction coordinate. In this way, dynamic force spectroscopy DFS exposes barriers – especially inner barriers – that are difficult or impossible to detect in solution assays. Because of the inherent logarithmic dependence of rupture force on speed of loading, the DFS method is most revealing when applied over many orders of magnitude in loading rate. Examining biomolecular bonds with dynamic force spectroscopy is leading to a new perspective of the important connection between force – time – chemistry in biology.

George Oster (University of California Berkeley) presented a talk entitled "How F1 ATPase uses nucleotide hydrolysis to generate a rotary torque." He discussed how the experimentally measured mechanical efficiency of the F1 ATPase under viscous loading is nearly 100%, far higher than any other hydrolysis driven molecular motor. A structural and bioenergetic analysis provides a molecular explanation for this remarkable property.

Session 2: Cellular Perspectives

These three talks [**Yale Goldman** (University of Pennsylvania), **Joyce Wang** (Boston University), and, **Charles Lindemann (**Oakland University)] and those by **Sheetz**, and **Elson** earlier in the meeting, have the common theme that the motor proteins and the cytoskeletal polymers exhibit bi-directional communication and energy transduction. The conventional energy transduction pathway is from metabolic energy into motion. Many cellular machines use energy liberated by splitting ATP or GTP to perform useful functions, such as motility, ion pumping, untwisting of tangled DNA or proof-reading of the genetic code during translation. It can easily be shown thermodynamically that this energy transduction implies an influence of the work output or mechanical properties of the load, such as its mass, stiffness or viscosity, on the rates of some of the accompanying biochemical reactions. In muscle, non-muscle myosin-based intracellular motility, locomoting cells and the flagellar axoneme the mechanical conditions, forces on the motors and properties of the substrate, strongly control the kinetics of the energy transduction process. Decoding the details of this 'reverse communication' and understanding the mechanisms at the molecular and atomic levels remain crucial tasks in most examples of cell motility.

Yale Goldman (University of Pennsylvania) showed several examples of the feedback of the loading conditions on actomyosin kinetics and some new methods for detecting the relevant mechanical and structural signals. This feedback is essential to minimize energy consumption. Non-muscle myosins participate in myriad cell biological roles, including development of the cell morphology, maintenance of native ultrastructure and signaling. Members of the myosin superfamily transduce force signals and move crucial cargoes to specific sub-cellular target. Wang used a new manipulatable substrate (cross-linked polyacrylamide) to detect traction forces of locomoting cells. He addressed the production of such forces, their magnitudes and mechanisms. The results are compatible with an engine-cargo model. How the cells detect and respond to mechanical properties of the substrate is just beginning to be understood. The functions of such detection may be probing the environment or long range signaling between cells or from the environment. **Lindemann** presented a model of the eukaryotic flagellum in which the transverse-force acting on the outer microtubule doublets regulates the dynein motors. A simulation based on this model replicates the behavior of cilia and flagella including mechanical sensitivity.

Force transduction by the force generators themselves controls their output and may also influence many other cellular processes. Another thread in these talks is that development

of new methods is essential to obtain discriminating experimental data. Using the widest possible armamentarium, including physical and engineering approaches, toward solving biological problems is the most fruitful avenue.

Session 3: Tissue/Organ Perspectives

The session on Biological Forces, Tissue-Organ Perspectives focused on the mechanisms of mechanotransduction in biological systems.

Shu Chien (University of California, San Diego) reported that the shear stress can activate integrins and a vascular endothelial growth factor receptor. The activation of these membrane proteins triggers intracellular signaling pathways to modulate gene expression and cellular functions. The temporal and spatial natures of the mechanochemical transduction in relation to flow dynamics may explain the preferential localization of atherosclerosis in branch points of the arterial tree.

Elisabeth Burger (Vrije University of Amsterdam) presented data showing that the flow of interstitial fluid in the canaliculi in strained bones induces significant shear stresses which are sensed by the osteocytes to induce bone remodeling. High bone strain and interstitial flow causes osteoblast recruitment and bone growth, whereas reduced bone strain and interstitial flow leads to osteoclast attack and bone loss. In addition to the modulation of cellular functions such as proliferation, motility, and secretion, mechanical forces also cause structural remodeling, e.g., the reorganization of cytoskeletal fibers and the alignment of endothelial cells and bone trabeculae and osteons with the direction of force application.

Stephan Levin (private practice) presented the tensegrity model of spine mechanics. In the tensegrity model, the bones act as compression elements enmeshed in soft tissues. In contrast to the traditional “stack of block” models, tensigrity structures are omni-directional, hierarchical, nonlinear, and independent of gravity, and local load distributing. The tensegrity model allows the synegistic linkage of structure and function for the creation of an integrated hierarchical system.

III. Conclusions and Recommendations

The problems in force transduction in biology, even the 'small' problems, are of such complexity that many different techniques and approaches are needed for progress. The challenge is assembling the right set of skills for a particular problem. However, once this is done, considerable progress can be expected. The NSF can play a very significant role in fostering the sort of interdisciplinary research to address these important problems.

The following comments summarize the major conclusions of the Workshop:

- Problems in the field of biological force transduction are not just a set of biochemical issues, but span the molecular to the macroscopic; thus, new engineering tools and models are needed.
- The study of force transduction has significant potential for development of new biomaterials, as mechanisms for force transduction are better understood.
- The mechanical issues in force transduction bear directly on issues inherent in the protein folding problem.
- The relation between the mechanical stress or shear state of a molecule and its functionality is still very poorly understood and represents an important challenge, and a great opportunity.
- The mechanical function of the cytoskeleton remains poorly understood and represents a significant challenge with a large reward once better understood.
- The complexity of the problems involved in force transduction demand extensive interdisciplinary interaction and will require the active collaborations among chemists, biologists, physical scientists and engineers.
- More collaboration between and theory and experiment is needed. Theoretical efforts lag experiment for the most part owing to issues of complexity. This represents a significant opportunity for new research.
- The “materials and mechanical” properties of biomolecules are major determinants in their biological function. This represents a new frontier for research, in which the NSF is ideally poised to make a major contribution.
- Because of the spread in possible impacts of research on biological force transduction, from fundamental science to clinical benefits, major opportunity exists for coordination and cooperation between NSF and NIH in jointly funding research programs.

NSF CONTACTS:

Hollis Wickman Division of Materials Research hwickman@nsf.gov	Soo-Siang Lim Division of Integrative Biology and Neuroscience Slim@nsf.gov
Eva I. Barak Division of Molecular and Cellular Biology ebarak@nsf.gov	Frederick Heineken Bioengineering and Environmental Systems Engineering Division Fheineke@nsf.gov
Kamal Shukla Division of Molecular and Cellular Biology kshukla@nsf.gov	Denise Caldwell Physics Division Dcaldwell@nsf.gov

Conference Participants

Barak, Eve ebarak@nsf.gov	NSF
Bausch, Andreas abausch@deas.harvard.edu	Division of Engineering and Applied Sciences Harvard University McKay Lab 5th Fl. Cambridge, MA 02138
Ben-Simon, David David.Bensimon@lps.ens.fr	Ecole Normale 24, rue Lhomond 75005 Paris FRANCE
Boal, David david_boal@sfu.ca	Dept. of Physcis Simon Fraser University Vancouver BS CANADA V5A 156
Braam, Janet braam@bioc.rice.edu	Dept. of Biochemistry & Cell Biology Rice University W200P George R. Brown Hall Houston, TX 77005
Brodland, G. Wayne brodland@sunburn.uwaterloo.ca	Dept. of Civil Engineering University of Waterloo Waterloo, ON, N@L 2G1 CAMADA
Bronkhorst, Philippine	University of Amsterdam Department of Molecular Cytology Amsterdam The Netherlands
Brownell, William E. brownell@bcm.tmc.edu web page	Department of Otolaryngology Baylor Medical School Neurosensory Center, NEUR A508 Houston, TX 77030
Bruinsma, Robijn	UCLA 2-240B Knudsen mail code: 154705 Department of Physics & Astronomy Los Angeles, CA 90095-1361
Burger, Elisabeth eh.burger.ocb.acta@med.vu.nl	Department of Oral Cell Van der Boechorst Straat 7 ACTA - Free University 1081BT Amsterdam Netherlands

Buxbaum, Robert buxbaum@pilot.msu.edu	Michigan State University 23 Giltner Hall East Lansing, MI 48824
Carter, Dennis R. dcarter@leland.stanford.edu	Stanford University Department of Mechanical Engineering Biomechanical Engineering Division Stanford, CA 94305
Chadwick, Richard S.	NIH/NIDCD intramural NIH Bldg 9 Rm 1E-116 9 Center 9 MSC 0922 Bethesda MD 20892
Chien, Shu shuchien@ucsd.edu/a>	Director Whitaker Institute of Biomedical Engineering UCSD La Jolla, CA 92093
Chin, Jean chinj@nigms.nih.gov web page	NIH-NIGMS Cell Biology & Biophysics 45 Center Drive Bethesda, MD 20892-6200
Cooke, Mary COOKE@surgery.wisc.edu	Department of Surgery University of Wisconsin Clinical Science Center-Core H5 BX3236 600 Highland Ave Madison, WI 53792
Cowin, Steven scccc@cunyvm.cuny.edu	Dept. of Mechanical Engineering City College of New York Convent Ave. at 138th St. New York, NY 10031
Davies, Peter F. pfd@pobox.upenn.edu	Professor and Director Institute for Medicine and Engineering University of Pennsylvania 1010 Vagelos Laboratories 3340 Smith Walk Philadelphia, PA 19104
Discher, Dennis discher@seas.upenn.edu	University of Pennsylvania 112 Towne Bldg. Philadelphia, PA 19104-6315
Elson, Elliot ELSON@BIOCHEM.WUSTL.EDU	Biochemistry & Molecular Biophysics Washington University Medical School Campus Box 8231 St. Louis, MO 63130
Evans, Evan evans@physics.ubc.ca	Boston University Biomedical Engineering 44 Cummington Street Boston, MA 02215

Fernandez, Julio fernandez.julio@mayo.edu	Mayo Clinic Foundation Dept. of Physiology and Biophysics 200 First St. S.W. Rochester, MN 55905
Flicker, Paula F. flickerp@nigms.nih.gov	NIH- NIGMS Bldg. 45, Rm. 2AS.13H 45 Center Dr. MSC 6200 Bethesda, MD 20892-6200
Forgacs, Gabor forgacsg@missouri.edu	Department of Physics Clarkson University P.O. Box 5820 Potsdam, NY 13699-5820
Frangos, John A. jfrangos@ucsd.edu web page	University of California Dept. of Bioengineering EBU I 6407 San Diego, CA 92093
Frey, Erwin frey@cmt.harvard.edu	Department of Physics Harvard University Jefferson Laboratory Cambridge, MA 02138
Fygenson, Deborah K deborah@physics.ucsb.edu	UCSB Department of Physics 4133 Broida Hall Santa Barbara, CA 93106
Gardel, Margaret gardel@physics.harvard.edu	Department of Physics Harvard University McKay Lab 5th Fl. Cambridge, MA 02138
Glazier, James jglazier@rameau.phys.nd.edu	Notre Dame Nieuwland Science Hall 316 Notre Dame, Indiana 46556
Goldman, Robert D. r-goldman@nwu.edu	Dept. of Cell Molec. Struct. Biology Northwestern University Medical School 303 E Chicago Ave / Ward 11-145 Chicago, IL 60611
Goldman, Yale goldmany@mail.med.upenn.edu	University of Pennsylvania Pennsylvania Muscle Institute D700 Richards Bldg. 3700 Hamilton Walk Philadelphia, PA 19104-6083
Grodzinsky, Alan alg@MIT.EDU	MIT 38-377 Division of Bioengineering & Environment Cambridge, MA 02139

Hammer, Dan	Department of Chemical Engineering University of Pennsylvania 120 HAYDEN/6316 220 South 33rd Street Philadelphia, PA 19104-6393
Hasenstein, Karl hasenstein@usl.edu	Univ. of Louisiana Department of Biology Lafayette, LA 70508
Heidemann, Steve heideman@msu.edu	Michigan State University Department of Physiology 108 Giltner Hall East Lansing, MI 48824-1101
Heiney, Paul heiney@dept.physics.upenn.edu	Dept. of Physics & AstronomyUniv. of Pennsylvania 2N24 David Rittenhouse Laboratory 209 South 33rd Street Philadelphia, PA 19104-6396
Hu, Yufang yufang@engineering.ucsb.edu	Chemical Engineering Dept UCSB Santa Barbara, CA 93106-5080
Humphrey, Joseph (Pepe) jah5nn@Virginia.EDU	Department of Mechanical & Aerospace Engineering School of Engineering and Applied Science University of Virginia 122 Engineer's Way P.O. Box 400746 Charlottesville, VA 22904-4746
Hunt, Alan ajhunt@umich.edu	Department of Biomedical Engineering 300 North Ingalls Building Room 962 University of Michigan, Ann Arbor, MI 48109-2007
Ingber, Don ingber@a1.tch.harvard.edu	Harvard Medical School Children's Hospital 300 Longwood Ave Surgery/Pathology Enders 1007 Boston, MA 02115
Islam, Mohammad F. islam@dept.physics.upenn.edu	University of Pennsylvania Department of Physics & Astronomy 209 S. 33rd Street David Rittenhouse Lab Philadelphia, PA 19104
Iwasa, Kuni H. iwasa@nih.gov	9 Center Dr MSC 0922 NIH Bldg. 9, Rm. 1E120 Bethesda, MD 20892-0922

Janmey, Paul janmey@mail.med.upenn.edu	Dept. of Physiology University of Pennsylvania 1010 Vagelos/6383 3340 SMITH WK Philadelphia, PA 19104-6383
Kamm, Roger rdkamm@mit.edu	MIT 3-260 Division of Bioengineering & Environment Cambridge, MA 02139
Kas, Josef	Physics Department Campus Mail Code: C1600 University of Texas Austin, TX 78712
Keller, Raymond rek3k@virginia.edu	Department of Biology Gilmer Hall University of Virginia Charlottesville, VA 22903
Knecht, Dave	Department of Molecular/Cell Biology University of Connecticut U-125 Storrs CT 06269-0001
Ko, Kevin kevin_ko@hotmail.com	University of Toronto MRC Group in Periodontal Physiology Rm. 243, Fitzgerald Building 150 College St. Toronto, Ont. CANADA M5S 3E2
Kolomeisky, Anatoly B. abk7@glue.umd.edu	University of Maryland
Kuo, Scot C. skuo@bme.jhu.edu web page	Biomedical Engineering School of Medicine Johns Hopkins University Rm. 724 Ross Research Building 720 Rutland Avenue Baltimore, MD 21205
Lal, Jyotsana jlal@anl.gov	Argonne National Labs. PNS 360 C-245 9700 S. Cass Avenue Argonne, IL 60439
Lee, James Chak-Man	University of Pennsylvania 3500 Powelton Ave. A112 Philadelphia, PA 19104
Lee, Juliet jlee@uconnvm.uconn.edu	Department of Molecular and Cell Biology University of Connecticut 75 N. Eagleville Rd. U-125, Room 311 Storrs, CT 06269-3125

Levin, Stephen smlevin@biotensegrity.com web page	Potomac Back Center 100 East Street SE Vienna, VA 22180
Lin, Keng-hui khlin@student.physics.upenn.edu web page	Dept. of Physics University of Pennsylvania 209 S. 33rd Street Philadelphia, PA 19104
Lindemann, Charles lindeman@oakland.edu web page	Oakland University Biological Sciences 330 Dodge Hall Rochester, MI 48309-4475
Lymn, Richard W. LymnR@exchange.nih.gov web page	Director, Muscle Biology Branch National Institute of Arthritis & Musculoskeletal & Skin Diseases National Institutes of Health Bethesda, Maryland 20892-2350
MacKintosh, Frederick M. fcm@umich.edu	Department of Physics 272 West Engineering University of Michigan Ann Arbor, MI 48109-1120
Mahadevan, L. l_m@mit.edu	MIT 3-246 Dept. of Mechanical Engineering Cambridge, MA 02139
Margulies, Susan margulies@seas.upenn.edu	Univ. of Pennsylvania Department of Bioengineering 105 Hayden Hall 3320 Smith Walk Philadelphia, PA 19104-6392
McGrath, James	Johns Hopkins 720 Rutland Ave Ross 724 Baltimore, MD 21009
Meaney, David dmeaney@seas.upenn.edu	Univ. of Pennsylvania Department of Bioengineering 105E Hayden Hall 3320 Smith Walk Philadelphia, PA 19104-6392
Mogilner, Alexis aimogilner@ucdavis.edu	UC Davis Department of Mathematics 682 Kerr Hall Davis, CA 95616
Mosher, Deane DFM1@MEDICINE.WISC.EDU	University of Wisconsin Medical Sciences Center 4459 1300 University Ave Madison, WI 53706

Newman, Stuart newman@nymc.edu	Department of Cell Biology & Anatomy Basic Sciences Building New York Medical College Valhalla, NY 10595
Nikolaides, Michael nikolaid@deas.harvard.edu	Dept. of Physics Harvard University McKay Lab 5th Fl. Cambridge, MA 02138
Oddou, Christian oddou@univ-paris12.fr	University of Paris
Oster, George goster@nature.Berkeley.edu	201 Wellman Hall University of California Berkeley CA 94720
Ou-Yang, H. Daniel hdo0@lehigh.edu web page	Department of Physics, Lehigh University 16 Memorial Drive East Bethlehem, PA 18015
Pao, Wen-Jung wjpao@sas.upenn.edu	Department of Physics and Astronomy University of Pennsylvania DRL 209 South 33rd St. Philadelphia, PA 19104
Photos, Peter J. photos@seas.upenn.edu	University of Pennsylvania 220 S. 33rd St. Dept of Chemical Engineering Rm 311A Towne Philadelphia, PA 19104-6393
Powell, Courtney	Brown University/Miriam Hospital Dept. of Pathology 164 Summit Ave. Providence, RI 02906
Raphael, Robert M. rraphael@bme.jhu.edu web page	Johns Hopkins University Department of Biomedical Engineering Traylor Bldg., Rm. 613 Johns Hopkins Univ. School Medicine 720 Rutland Ave. Baltimore, MD 21205
Roy, Partha roy@email.unc.edu	University of North Carolina
Sachs, Frederick sachs@buffalo.edu	SUNY-Buffalo 124 Sherman Hall Physiology and Biophysics Buffalo, NY 14214

Schneider, Victor S. vschneider@hq.nasa.gov	NASA Life Sciences Division/ULA NASA Headquarters 300 E Street, SW Washington, DC 20546
Schulten, Klaus kschulte@ks.uiuc.edu	University of Illinois Urbana/Champaign Department of Physics 313 Loomis MC 704 1110 West Green St, Urbana, IL 61801
Shah, Jagesh V. jvshah@ucsd.edu	University of California - San Diego 9500 Gilman Drive CMM-E 3072, Mail Code 0660 San Diego, CA 92093-0660
Sheehan, Maureen msheehan@seas.upenn.edu	University of Pennsylvania Towne 311A 220 S. 33rd St. Philadelphia, PA 19104
Sheetz, Michael ms2001@columbia.edu	Duke University 388C Nanaline H Duke Box 3709 Medical Center Durham, NC 27710
Skalak, Tom tcs4z@Virginia.EDU	University of Virginia BME PO Box 800759 Charlottesville, VA 22908-0759
Steinberg, Marcia steinbem@csr.nih.gov	NIH Center for Scientific Revie 6701 Rockledge Drive Room 5140, MSC 7840, Bethesda, MD 20892-7840
Suki, Bela bsuki@bu.edu	Department of Biomedical Engineering Boston University 44 Cummington Street Boston, MA 02215
Sykes, Cecile cecile.sykes@curie.fr	Institut Curie Paris
Tang, Jay X. jxtang@indiana.edu	Indiana University Department of Physics Swain West 165 727 East Third St. Bloomington, IN 47405

Tranquillo, Robert T. tranquil@tc.umn.edu	University of Minnesota 151 Amundson Hall 421 Washington Ave SE Minneapolis, MN 55455
Urry, Dan danurry@uab.edu	Bioelastics Res. Ltd. 2800 Milan Ct. Suite 386 Birmingham, AL 35211
Usami, Shunichi usami@bioeng.ucsd.edu	Department of Bioengineering and The Whitaker Institute of Biomedical Engineering University of California, San Diego 9500 Gilman Drive La Jolla, CA 92093-0427
Valentine, Megan mvalenti@deas.harvard.edu web page	Dept. of Physics Harvard University 5th Fl. McKay Lab Cambridge, MA 02138
Vandenburgh, Herman H. Herman_Vandenburgh@brown.edu	Brown University/Miriam Hospital Dept. of Pathology 164 Summit Ave. Providence, RI 02906
Veldhuis, Jim jveldhuis@uwaterloo.ca	University of Waterloo Dept. of Civil Engineering 200 University Ave., West Waterloo Ontario N2L 3G1 Canada
Wang, Hongyun hongwang@cse.ucsc.edu web page	Department of Applied Mathematics and Statistics Jack Baskin School of Engineering University of California 1156 High Street Santa Cruz, CA 95064
Wang, Yu-Li YuLi.Wang@umassmed.edu	UMass Medical School 55 Lake Avenue North Worcester, MA 01655
Weiss, Paul S. stm@psu.edu web page	Penn State Chemistry Department 0152 Davey Laboratory University Park, PA 16802
Weitz, David A. weitz@deas.harvard.edu web page	Dept. of Physics & Division of Engineering and Applied Sciences Harvard University Pierce Hall 231 29 Oxford St. Cambridge, MA 02138

Wendling, Sylvie wendling@univ-paris12.fr	Universités Paris 12 - Paris 7 CNRS ESA 7052 Centre Multidisciplinaire de Créteil Faculté des Sciences et Technologie LMP - Laboratoire de Mécanique Physique 61, avenue du Général de Gaulle 94010 Créteil Cedex France
Wong, Joyce jywong@bu.edu	Boston University ENG Biomedical Engineering 44 Cummington Street Boston, MA 02215
Zabow, Gary gary@atom.harvard.edu web page	Harvard University Physics Department Harvard University, Physics Dept. 17a Oxford Street Cambridge, MA 02138
Zhang, Fan fanzhang@student.physics.upenn.edu	University of Pennsylvania 209 south 33rd st. Dept of Physics Philadelphila, PA, 19104

The National Science Foundation (NSF) funds research and education in most fields of science and engineering. Grantees are wholly responsible for conducting their project activities and preparing the results for publication. Thus, the Foundation does not assume responsibility for such findings or their interpretation.

NSF welcomes proposals from all qualified scientists, engineers and educators. The Foundation strongly encourages women, minorities, and persons with disabilities to compete fully in its programs. In accordance with federal statutes, regulations, and NSF policies, no person on grounds of race, color, age, sex, national origin, or disability shall be excluded from participation in, be denied the benefits of, or be subjected to discrimination under any program or activity receiving financial assistance from NSF (unless otherwise specified in the eligibility requirements for a particular program).

Facilitation Awards for Scientists and Engineers with Disabilities (FASED) provide funding for special assistance or equipment to enable persons with disabilities (investigators and other staff, including student research assistants) to work on NSF-supported projects. See the program announcement or contact the program coordinator at (703) 292-6865.

The National Science Foundation has Telephonic Device for the Deaf (TDD) and Federal Relay Service (FRS) capabilities that enable individuals with hearing impairments to communicate with the Foundation regarding NSF programs, employment, or general information. TDD may be accessed at (703) 292-5090 or through FRS on 1-800-877-8339.

The National Science Foundation is committed to making all of the information we publish easy to understand. If you have a suggestion about how to improve the clarity of this document or other NSF-published materials, please contact us at plainlanguage@nsf.gov.