Lighting Up The Mechanome

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The Genome and general "omics" approaches, such as the proteome, interactome, and phosphatome, among others, are powerful system level tools in biology. Each of these fields is supported by advances in instrumentation and computation, including DNA sequencers and mass spectrometers, which, for example, have been critical in "sequencing" the genome. Revolutionary advances in biology have also played a critical role in developing these tools by harnessing the machinery of the polymerases to copy DNA or using RNA interference to capture and turn off genes. Biophysics and biomechanics research likewise has provided significant advances in experimental methods and microscope development bringing new resolution to molecular and cellular scale measurements. Despite these advances, a systems level "omics" perspective has yet to be developed to describe the general role of force, mechanics and machinery in biology. Although the mechanome hasn't been "sequenced," it shares the promise of the genome in that a better understanding of biological machinery will provide new strategies and targets for fighting disease.

Many diseases are mechanical in nature. Malaria, which infects 500 million people a year resulting in 2 million deaths each year, acts through a number of mechanical processes, including parasite invasion into the red blood cell (RBC), increased RBC adhesion and overall stiffening of the RBC. Disease progression ultimately leads to an infected cell's impaired ability to squeeze through capillaries, rupture of the RBC membrane and the release of newly minted malaria parasites. (E1)

Cancer is also a leading cause of death. In addition to the machinery of viral infection responsible for Hepatitis B, Human papillomavirus, and related cancers, the progression of cancer exhibits many mechanical manifestations. The process of metastasis involves detachment of tumor cells from the initial growth site, migration, circulation and subsequent invasion through re-attachment of cancerous cells to new locations. Furthermore, intracellular changes in cancerous cells bring about cell growth and proliferation, cell motility and migration. Such processes are targeted by one of the most potent chemotherapy drugs, taxol, which impedes cancer progression by stabilizing microtubules in a polymerized state and thus shutting down the machinery of cell division.

The Human Genome is contained within approximately 3 billion base pairs, bp, of DNA. Each cell contains a length of DNA that if connected end-on-end, would roughly span the length of one's outstretched arms. DNA is quite flexible compared to other cellular filaments, but despite this flexibility, our DNA is compacted in highly ordered structures through multiple levels of hierarchy, in order to fit DNA into cells. (E2) In addition to conventional Watson and Crick base pairing, forces and interactions in DNA include histone protein-DNA nucleosome complexes, chromatin fibers, chromatids and ultimately 46 chromosomes. Our biological structures in general contain many levels of structural hierarchy and are highly organized.

Molecular machinery: From our DNA sequence, biological motors, including the DNA and RNA polymerases and the ribosome, make RNA and ultimately proteins through processes that couple mechanical and chemical steps. This machinery is impressive as much of the copying and translating is done with great accuracy. The

ribosome, a huge motor spanning 20 nm that is actually composed of about 65% ribosomal RNA, assembles proteins from messenger RNA. This machinery is also critical, and many antibiotics target ribosomal translation in bacteria.

Other molecular motors such as myosin and kinesin run on different cytoskeletal tracks including actin filaments and microtubules respectively.(E3) Among these motors, myosins are responsible for skeletal and cardiac muscle contraction and contribute to cell migration and cell division processes such as contractile ring closure. Similarly, kinesins are important actors in transport and cell division. Kinesins are small, processive and run towards the plus (fast polymerizing) end of microtubules. In some cases, such as the kinesins that carry vesicles containing neurotransmitters along the sciatic nerve axon, this progress takes place over the length scale of a meter and is a critical mechanism for cargo delivery. (E4)

Nature's design of biological motors can be classified in a common way by cataloging the motor's general structural features, fuel type, stepping distance, stall force and other mechanical parameters. Detailed measurements of the motility cycles and underlying mechanisms for motility provide still more insight. (E5) Ultimately, "sequencing" the mechanome will allow for discovering the design features for motors in general, to outline the rules that govern biological motor machinery, and to catalog biological machinery.

A remarkable feature arises when comparing the family trees of kinesins and myosins. A range of structural forms are found including single, double and tetramer headed members with various lengths connecting the motor and cargo binding domains. Similarities also exist at the molecular level as both kinesin and myosin are fueled by

hydrolysis of ATP, and share common structural elements such as the binding pocket for ATP (Vale, 1996). Despite the different tracks and overall purpose for motility, there are common biological machinery designs that have been optimized. It appears that Nature has copied and pasted design elements for motors that have similar roles and physical constraints. Another example of a common motor design being used on different motility tracks is in the triple AAA+ motors where similarly structured motors run on a range of track types such as microtubules, DNA and peptides and are involved in a number of critical cellular processes (Ogura and Wilkinson, 2001).

Cellular machinery: Cellular machinery, for example the processes of cell division, wound healing and migration, can also be classified by general design principles. Forces originating from many sources, such as biological motors, polymerization of filaments, molecular conformational transitions and fluid pressure can act on structures including cytoskeletal filaments, membrane barriers, receptor ligand linkages and protein complex adhesion sites. Among these interactions, cell machinery typically occurs in cycles such as migration, processes of extension, adhesion, contraction and detachment. Highly tuned control over these forces and structures is orchestrated through both mechanical and biochemical signals that initiate and terminate motility cycles. Mechanotransduction is a critical aspect of this control and refers to the process where cells actively respond to mechanical signals through changes in morphology, signaling and biochemical response.

Migration of the neuronal growth cone exhibits a range of interesting cell machinery. The growth cone axon has a main core structure that consists of bundled microtubules. At the tip of this bundle, actin filaments are structured in both bundled

finger like spikes known as fillopodia, and web like flattened sheets called lamellipodia. Actin filaments can be arranged in a variety of higher level structures and these are coordinated by a series actin binding proteins (ABPs). The overall structures of these ABPs also vary widely, yet many share a common actin binding domain. In cell division, an actin based ring-like bundle contracts to separate the cell into the progeny cells. This processes, called cytokinesis, shows design elements that appear to be cut and pasted. For example in cell division of higher plants, a similar process generates a microtubule based structure at the cell division locus (Jurgens, 2005). Even bacteria contain tubulin and actin homologues, namely FtsZ and MreB, respectively, that form higher level structures and machinery that resembles their counterparts such as microtubules (MreB) and formation of the cytokinetic ring in bacteria (FtsZ) (Erickson, 1998, van den Ent, et al., 2001).

Tissue and higher level machinery: Mechanics play a critical role in tissue and higher level scales. In fact, the mechanics of a cells environment is an important factor in determining its fate. Stem cells plated on different stiffness substrates differentiate into different cell types taking on properties of tissues similar to their mechanical environment. For example, mesenchymal stem cells grown on a matrix with elasticity resembling tissues such as 1kPa brain, 10kPa muscle and 100kPa bone substrates differentiate into cells resembling neurons, muscles and osteoblasts respectively (Engler, et al., 2006).

The external environment of the cell is also rich with organized mechanical structures, as a typical extracellular matrix is composed of cable-like collagen molecules, rubber band-like elastin molecules and sponge-like proteoglycans. For example, tendon contains collagen organized into a bundle form with many levels of hierarchy, including

mineralization. Tendon is designed for low strain and fatigue and can sustain many cycles of loading and unloading over a long timespan. The failure of tendon is a common sports injury. In contrast, the dense collagen network of the chorioamnion membrane surrounding a fetus is designed to eventually fail, though early failure in this structure is responsible for one third of all premature births (Oyen, et al., 2005). Mechanical forces may also affect the organization of structures. Bone requires mechanical stimulation to maintain density and collagen stress fibers will align in the direction of maximal load. Many higher level systems are mechanical, such as muscular-skeletal, respiratory, cardiovascular, lymphatic, integumental etc.

Probing the molecular to cellular mechanome: We are poised with a range of advanced instrumentation and experimental methods to measure the mechanome at the molecular level. For example, force microscopy, where controlled loads are applied to structures while they are tracked with nanometer level resolution, can be used to measure mechanical transitions in unfolding protein structures, rupture of individual molecular interactions, or watch the motility of biological motors.

The forces required in these measurements transverse a huge range. At the molecular level a balance is obtained between stabilizing an interaction against thermal energy, kT, while maintaining the ability to break an interaction through transition state barriers of a few kT. Given that kT is on the level of 4 pN-nm, or 4.1 x10^{-21} J, and typical transition distances on the length scale of nanometers, forces ranging from a pN to a few tens of pN are ideal for probing molecular machinery. ATP hydrolysis is the energy source for many biological motors and provides about 20-25 kT of energy. Unfolding proteins requires more force, on the scale of tens to hundreds of pN. Furthermore, cell

level machinery may involve many molecular interactions and can require force magnitudes on the scale of nN and larger.

A broad range of complementary force probe methods span these force ranges including the atomic force microscope (AFM), micropipette manipulation, magnetic traps, microfabricated cantilevers and the optical trap.(Suresh, 2007) Combinations of these force probes, such as combined optical trapping and magnetic particle manipulation, are very powerful for extending the force range. The optical trap has been a mainstay technique in single molecule biophysics research and is an ideal tool for probing the molecular interactions of biological motors and receptor-ligand interactions. Fluorescence microscopy is also a powerful method for observing single molecules. Advances in this technology have been used to track fluorophore labeled biological motors with nanometer level resolution (Yildiz, et al., 2004). Energy transfer methods using pairs of fluorophores, called FRET, provides a fluorescence based ruler with a $1/r^6$ dependence, on the length scale of nanometers which is useful for monitoring conformational changes.

Recently, these two mainstay single molecule biophysics techniques, optical traps and single molecule fluorescence, FRET, have been combined on a single platform (Tarsa, et al., 2007). The combination of these methods marries the power to mechanically interrogate and control structures while observing them with fluorescence. This combined method has been demonstrated using a model system consisting of a DNA based hairpin that can be opened and closed with about 15 pN of force from the optical trap (Figure 1). A FRET pair was placed at the base of the hairpin to report whether the hairpin was open or closed through high and low FRET states respectively. Many

additional advances such as light based methods of laser scissors, photobleach recovery, triggering, uncaging and time resolved measurements have been developed and can be integrated to produce powerful new tools.

Advances in assay development, molecular biology and linkage chemistry are also critical to the development of the field. Molecular biology strategies for genetically labeling proteins such as with GFP have been very powerful. New probes, such as enhanced organic dyes and quantum dots, allow for single molecule observations over extended time periods. Molecular biology can also be used to create physical handles for physical manipulation, exploiting for example the aptamer interactions and tethers based on the M13 phage (Khalil, et al., 2007).

With these tools and advances in assays we can begin to probe Nature's machinery. Ultimately, we hope to gain quantitative understanding of molecular and cellular machinery and measure the forces and strength of the individual mechanical steps that underlie the mechanome. By developing an understanding the physical rules that govern biological systems and measuring Nature's machinery we can "sequence" the mechanome.

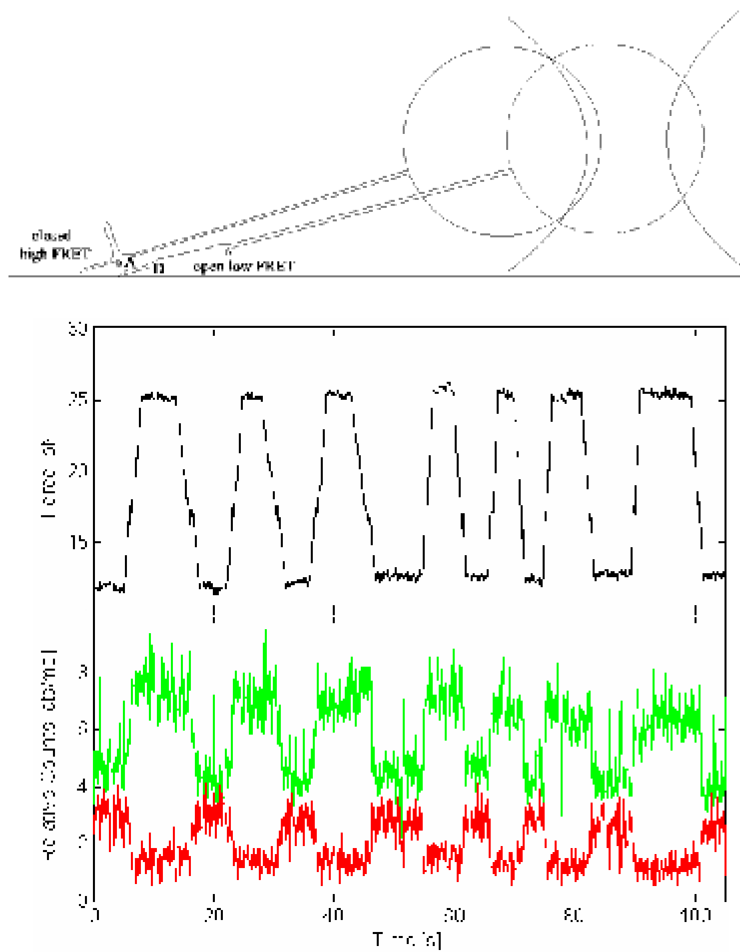

Figure 1. A cartoon shows the experimental design with open and closed states of a DNA hairpin complex that is controlled with force from an optically trapped bead. A donor and acceptor FRET pair is placed at the base of the hairpin to monitor whether it is open or closed. Force on the hairpin is repeatedly transitioned from low to high with hairpin opening occurring at approximately ~18pN. Photon counts are shown in the lower frame with donor and acceptor FRET signals (red and green respectively) changing simultaneous with the hairpin opening and closing transitions.

Endnotes:

E1. The RBC circulates in the body about half a million times over a 120 day period. Cancer and Malaria were topics of the last two recent GEM4 summer schools. http://www.gem4.org/

E2. Human cells are typically 10-30μm in diameter with the smallest, sperm, only a few microns in diameter to the largest, the egg, at ~100μm in diameter. The size of randomly packed DNA can be estimated from the radius of gyration which goes as $b \cdot N^{1/2}$, where N is the number of segments and b is the length of each segment. DNA has a length of 0.3nm/bp, a persistence length of 50nm. An average chromosome may contain 1.3×10^8bp of DNA yielding a radius of gyration of 44μm, larger than the typical cell size. Chromosomes were first observed by Karl Wilhelm von Nägeli, in 1842.

E3. Mechanically these filaments are impressive. Actin filaments have a Young's modulus of 2.3 GPa and microtubules have a persistence length on the order of mm.

E4. Kinesin has a velocity of 800nm/s and traveling the sciatic nerve might take about half a month. Diffusion alone would take much longer than a lifetime. This estimate can be obtained from the Einstein-Sutherland-Smoluchowski relation where a 1μm diameter cargo has a diffusion constant of $D=kT/6\pi\eta a=2\times10^{-9}$ cm^2/s. Diffusion time is proportional to the distance squared $x^2/D=7*10^4$ years.

E5. Kinesin for example has been extensively characterized. It has a stepping distance of 8nm for each ATP molecule consumed, steps occur upon ATP binding, kinesin takes about 100 steps/second, runs at 800nm/second, runs for about 1μm before loosing hold of its track, coordinates the action of the two heads, shuts down its hydrolysis in the absence of cargo, stalls at a load of around 5-6 pN and walks much like we do.

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