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ACTIVE BIOMIMETIC SYSTEMS: FORCE GENERATION AND CARGO TRANSPORT BY MOLECULAR MACHINES

REINHARD LIPOWSKY* and ANGELO VALLERIANI
Department of Theory & Bio-Systems, MPI of Colloids and Interfaces†
Science Park, 14424 Potsdam, Germany
**reinhard.lipowsky@mpikg.mpg.de*

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This special issue of Biophysical Reviews and Letters describes recent advances in the area of active biomimetic systems, which are inspired by the cytoskeletal architecture found in all eukaryotic cells. The main building blocks of these systems are provided by two types of cytoskeletal filaments, F-actin and microtubules, as well as molecular stepping motors such as kinesins and myosins. All of these building blocks represent molecular machines: They are coupled to nucleotide hydrolysis and are able to convert the chemical energy released from this process into mechanical work. Bundles of filaments and teams of stepping motors generate strong pushing forces and perform long-ranged cargo transport.

Cytoskeletal Architecture as a Source of Inspiration

One astounding aspect of eukaryotic cells is their ability to undergo dramatic morphological transformations: they can adapt their shape in order to squeeze themselves through very narrow pores, they can extend long "feet" in order to crawl along surfaces, and they can divide themselves up into two daughter cells. All of these transformations are accomplished by reconstructing the cytoskeleton of the cells at the molecular level.

The cytoskeleton of eukaryotic cells is built up from two different types of building blocks: (i) Cytoskeletal filaments, primarily F-actin and microtubules, and (ii) Molecular stepping motors such as kinesins and myosins. Actin filaments and microtubules are very thin and fairly long rods, which form by self-assembly of actin monomers and tubulin dimers, provided the corresponding concentrations exceed certain threshold values. Stepping motors are dimeric proteins with two motor domains or "heads" that are used as "legs" or "lever arms".

Cytoskeletal reconstruction involves two rather different processes: (i) Assembly and disassembly of actin filaments and microtubules; and (ii) Mutual displacements

†URL: http://www.mpikg.mpg.de/th

of interacting filaments and stepping motors. Both types of processes are coupled to nucleotide hydrolysis and convert the released chemical energy into mechanical work. Thus, both growing filaments and stepping motors represent molecular machines.

Basic Aspects of Actin Filaments and Microtubules

Actin filaments are formed by self-assembly of actin monomers, which have a size of 5 nm. These filaments have a helical structure with a thickness of 8 nm and can grow up to 20 μm. Microtubules are built up from tubulin dimers, which have a linear extension of 8 nm. The tubules usually consist of 13 parallel strands or protofilaments, have a thickness of 24 nm, and can be as long as several hundreds of μm. Both types of filaments are polar and have two different ends, a "plus" and a "minus" end, reflecting the polar character of the building blocks.

The assembly or polymerization of cytoskeletal filaments is coupled to nucleotide hydrolysis. Each actin monomer has one nucleotide binding pocket, by which it can bind a single ATP molecule and hydrolyze it into ADP. Likewise, each tubulin dimer has one such pocket, by which it can bind a single GTP molecule and hydrolyze it into GDP.[a] The rate of ATP and GTP hydrolysis is very small for actin monomers and tubulin dimers in solution but is strongly increased as soon as these monomers and dimers are incorporated into filaments.

This coupling between filament polymerization and nucleotide hydrolysis leads to nonequilibrium states that can be observed on the micrometer scale using optical microscopy. Actin filaments undergo treadmilling, i.e., they grow at their "plus" (or barbed) end and simultaneously shrink at their "minus" (or pointed) end. Microtubules, on the other hand, exhibit dynamic instabilities with relatively slow growth interrupted by relatively fast shrinkage. Growing filament ends exert pushing forces when they are in contact with membranes or other types of surfaces.

Basic Aspects of Stepping Motors

Stepping motors such kinesins have two "heads", by which they can bind to the filaments. Each head contains (at least) one nucleotide binding pocket for the binding and hydrolysis of a single ATP molecule. The kinesin motor exhibits tight coupling, i.e., it hydrolyzes one ATP molecule per mechanical step along the filament. Kinesin walks in a "hand-over-hand" fashion, i.e., by alternating steps, in which one head moves forward while the other one remains bound to the filament. Each step corresponds to a motor displacement of 8 nm corresponding to the lattice constant of the microtubule.

If there is no shortage of ATP, the motor makes about 100 steps in one second which leads to a velocity of about one micrometer per second. The absolute value

[a]The tubulin dimer has a second binding pocket for GTP that has no catalytic activity.

of this velocity is not impressive, but relative to its size, the motor molecule moves very fast: On the macroscopic scale, its movement would correspond to an athlete who runs 200 meters in one second! This is even more surprising if one realizes that the motor moves in a very viscous and noisy environment since it steadily undergoes thermally excited collisions with a large number of water molecules.

Because of this thermal noise, a single motor unbinds from the filaments after a certain run time and run length. For kinesin, the run time is about 1 s and the run length is about 1 μm. On length scales that are large compared to the run length, a single motor undergoes composite walks consisting of directed stepping interrupted by diffusive motion.

The run length of single motors is rather small compared to the long distances over which cargo particles such as vesicles or organelles are transported in eukaryotic cells. One extreme example is provided by axons that represent tube-like protrusions of nerve cells and can be as long as several meters. Fast transport over such macroscopic distances is achieved by teams of several motor molecules that pull on the same cargo particle in a cooperative manner. The cargo can be many micrometers in size and, thus, much larger than the motors themselves.

Multiscale Force Generation

Because of their small size, single filaments and single stepping motors generate rather small forces which are of the order of a few picoNewtons (10^{-12} N). In addition, a single molecular machine is rather sensitive to its environment and is easily perturbed by thermal noise, i.e., by collisions with the surrounding molecules. It is quite remarkable, however, that *all forces generated by living cells and organisms arise from the combined action of large numbers of such molecular machines.*

In this way, single cells can exert forces in the nanoNewton (10^{-9} N) range, and animals can generate forces of hundreds of Newtons. Thus, these molecular machines are able to bridge the gap between the nano- , micro- and macro-regimes and to generate a wide range of forces between a few picoNewtons and several hundreds of Newtons, i.e., over 14 orders of magnitude! In order to mimic and understand this amazing ability, one must develop biomimetic systems that contain bundles of filaments and/or teams of motors.

Overview over this Issue

The articles collected in this issue describe recent advances in our understanding of force generation and cargo transport by molecular machines as accomplished within the European Research Network on "Active Biomimetic Systems" (STREP Contract No. NMP4-CT-2004-516989) that was funded by the European Commission from 2005 until 2008.

The first three contributions address different filament architectures without stepping motors. The article by Romet-Lemonne *et al.* describes the force generation

of thick bundles of actin filaments and their site-directed nucleation by surface-anchored molecules. The second contribution by Harasztia *et al.* explores different methods to construct bundles and meshworks of actin filaments on pillared surfaces and in arrays of laser traps. Force generation by microtubules is discussed by Husson *et al.* who construct bundles of these filaments and use optical tweezers in order to measure the generated forces. One common theme of the latter two contributions is the effect of additional molecules that act as crosslinkers between the filaments.

The next two contributions are about the molecular mechanics of kinesin bound to microtubules. The kinesin/microtubule interactions are studied by Soncini *et al.* using molecular modelling and are shown to depend on the state of the nucleotide binding pocket of the molecular motor. The molecular structure of kinesin's motor head is also addressed from the experimental side by Kalchishkova and Böhm, who use recombinant methods to modify this structure and to elucidate the interplay between the nucleotide binding pocket and the microtubule binding domain.

Kinesin is also the main player in the contribution by Lipowsky *et al.*, who review the molitily of this motor that covers many length scales, from single mechanical steps with a step size of a few nanometers to transport of cargo particles over centimeters or even meters. One important aspect of the kinesin motor is that it is governed by a network of several motor cycles or chemomechanical pathways, which act as a gearbox for the motor. Other important aspects are its ability to work in crowded environments and to bypass obstacles on the microtubule. The latter situation is studied experimentally in the contribution by Dreblow *et al.* When immobilized on a structured surface, kinesins pull microtubules over this surface with a gliding velocity that can be controlled by the motor density as shown by Blümmel *et al.*, who use regular arrays of gold nanodots to construct regular arrays of pulling motors.

In vivo, teams of kinesin motors are involved in the transport of cargo particles over long distances up to centimeters. In many cases, the kinesin team competes with a team of dynein motors that try to pull the cargo particle in the opposite direction. As reviewed by Lipowsky *et al.*, such a tug-of-war represents a rather general molecular mechanism for the bi-directional transport of cargo as frequently observed in eukaryotic cells.

Teams of kinesin motors are also able to pull membrane tubes out of large unilamellar vesicles, a biomimetic system that is described by Campas *et al.* both experimentally and theoretically. For some parameter values, the tube is found to undergo oscillations in its length. The last contribution by Smith *et al.* describes several biomimetic systems by which one can study the interplay between actin filaments and myosin motors. One rather interesting possibility is to use these two building blocks in order to construct synthetic nano-muscles.