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Review Article

BIOLOGICAL MOTORS IN LIVING SYSTEMS

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ABSTRACT
Biological motors in living systems, from human beings to bacteria, are responsible for movement at molecular level as well as cellular level. These movements form the very basis of life, without which existence is not possible. The size of these motors is much smaller (about 1,000 to 100 times smaller) than any existing devices. They are biocompatible and do not need any enormous external power source, because they utilize the energy of ATP present in their ambient aqueous environment. Molecular motors consist of two functional domains, which are head domain containing the ATPase and microtubule-binding motor activity and nonmotor tail domains. They move unidirectionally along actin filaments or microtubules and compete with each other for the transportation of cargo within the cell. Inspite of various limitations, different studies have been undertaken to develop nanomachines based on these biological motors to tap their vast potential.

KEY WORDS: Motors, ATPase, unidirectional, microtubules.

INTRODUCTION

Combination of efficiency, reliability, and ubiquity of biological systems inspires new branches of fundamental and technological research. A number of synthetic model systems like dendrimers, molecular aggregates, and molecular motors mimic aspects of light harvesting, energy transfer, and motional processes found in nature. Movement, in one form or another, is an essential feature of all life at both the macroscopic and cellular level. Organisms, from human beings to bacteria, move to adapt to changes in their environments, navigating toward food and away from danger. By evolutionary modification over billion of generations, living organisms have perfected a group of biological nanomachines, structures, and processes. Cells, themselves, are assemblies of moving proteins, nucleic acids, and organelles. Therefore, life is made possible by the action of a series of biological nanomachines in the cell machinery.

Molecular motors or motor proteins are poly-peptide in nature. They convert chemical energy to mechanical work at a molecular scale inside the cells and have the ability to move molecules. Because of this unique property, they are responsible for various mechanical roles in living organisms. Living cells contain a large number of molecular motors: membrane pumps, cytoskeletal motors, growing filaments, and assemblers such as polymerases and ribosomes. Biological motor molecules such as the cytoskeletal motors kinesin and myosin represent powerful nanomachines (Khataee and Khataee, 2009). They are responsible for powering the transport of organelles and other molecular constituents, as well as the motility and contraction of entire cells. The size of these motors is much smaller (about 1,000 to 100 times smaller) than any existing devices. They are biocompatible and do not need any enormous external power source, because they utilize the energy of ATP present in their ambient aqueous environment.

Molecular motors in biological system

There are several classes of molecular motors which fulfill different functions in the living system. Intracellular motion was first observed in the alga Chara by Bonaventura Corti in the late 18th century, and chromosome movements were documented with remarkable accuracy in 19th century. With the aid of advanced microscopic techniques, visualization of cytoplasmic movements becomes possible to the finest details.

The bacteriophage (a virus that infects bacteria) possesses a rotary motor used to pack DNA into the bacteriophage head (Smith *et al.*, 2001). This motor works rather like a cork and corkscrew, where the DNA is the corkscrew (Simpson *et al.*, 2000). Simple but elegant, the molecular motor, consisting of a 10 nm ring of proteins, compresses DNA into the phage head by reducing the volume occupied by the DNA approximately 6000 fold; the resulting internal pressure within the phage head is thought to be of the order of 60 atmospheres.

Bacteria have rotary motors that drive the whip-like motion of their flagellae which, in turn, provide the bacteria with a swimming movement (Ryu *et al.*, 2000). These motors provide one of the best examples of self-assembly in nature. They comprise ~40 proteins (Suzuki *et al.*, 2004; Samatey *et al.*, 2004) that are synthesised within the cell and then transported, through the self-assembled structure of the motor, to the appropriate site for assembly of the flagellum on the outside of the cell. This type of self-assembly is frequently observed in biological systems and provides a blueprint for the requirements of nanodevices— they will have to be able to self-assemble at precise locations (Zhang, 2003).

The ATP Synthase motor is a rotary motor that exists in two parts (FoF1-ATPase), one is buried in the mitochondrial membrane (Fo), while the other part is attached to the central spindle of the membrane motor and is spun in vivo by movement of the membrane motor (F1).

The Fo motor is driven by a proton flux across the membrane and its movement leads to rotation of the F1 component. The lower motor then converts this mechanical energy into chemical energy that is used to synthesise ATP (which is the fuel of many other motors) (Table 1) (Youell and Firman, 2007). However, both motors are reversible and consumption of ATP can produce a proton pump that will pump protons out of the cell, across the membrane (Fillingame *et al.*, 2000; Stock *et al.*, 1999; Yasuda *et al.*, 2001; Sabbert *et al.*, 1996; Gao *et al.*, 2005).

Muscle (myosin) is a typical linear motor in that it enables sliding of actin fibres along myosin fibres, although the motion at the heart of the myosin motor is in part a rotary motion that is transmitted to the actin fibre as a linear motion through a long lever-arm. This lever- arm also amplifies the motion produced by the molecular motor from a few nanometres to 10 nm (Yanagida *et al.*, 2000). Myosins are ubiquitous in the cell, with a wide range of functions ranging from control of balance in complex organisms such as man, through to cell division during mitosis. Myosins in muscles work in ensembles and collectively displace actin filaments.

Kinesins represent the most useful type of linear motors as nature already uses them for carrying objects around the cell (Jia *et al.*, 2004). These motors travel along microtubules, which radiate around the cell in three dimensions, transporting their cargoes to various parts of it. The cargoes can be proteins, vesicles or organelles many times the size of the motor (Sheetz, 1999; Steinberg, 2000; Kamal & Goldstein, 2002; Seog *et al.*, 2004).

Dyneins also act as ATP-driven molecular motors that are able to generate a force relative to microtubules. They are placed in three classes, which are primarily determined by subcellular localization—inner- and outer-flagella-arm dyneins and cytoplasmic dyneins. This localization matches the cellular activities, the first being the inner- and outer-flagella-movements and the second is the movement of cellular organelles (Harrison & King, 2000).

Another important group of motors are those that utilize DNA as their linear track (rather like kinesin and dynein use microtubules) and these include polymerases such as RNA polymerase (Harada *et al.*, 1999), DNA polymerases, helicases and topoisomerases. One key area of interest generated by these molecular motors is single molecule DNA sequencing (Meldrum, 2000).

Apart from these, an unusual group of motors that move DNA, are DNA translocases (Seidel *et al.*, 2004). Unlike the other motors mentioned above, these enzymes do not simply run along the DNA track, but rather bind the DNA and pull the rest of it through the bound complex. This provides for a very flexible system because the motors usually have a specific recognition sequence on the DNA (therefore, the position on the DNA can be readily defined) and they can produce useful work, because they create relative motion with respect to the surface on which the DNA is attached. This multi subunit motor acts as the "cork" on a DNA "corkscrew" and winds the DNA into the bacteriophage head.

Molecular motors consist of two functional domains, which are head domain containing the ATPase and microtubule-binding motor activity and nonmotor tail domains. Motor domains are generally similar within the kinesin superfamily, with 30-40% sequence identity and have very different properties such as velocity and direction on movement. Nonmotor tail domains share little or no sequence similarity between different kinesin classes and have very different structures and associated subunits (Gindhart, 2006).

In our cells as well as in those of all animals and plants, three types of proteins are used for cargo transport: kinesins, dyneins and myosins (Schliwa and Woehlke, 2003). These motors have usually two heads, which they use as legs and by which they can bind to the filaments. In their bound states, these molecular motors undergo a cyclic sequence of conformational transitions, a so-called motor cycle, that enables them to transform the chemical energy of single adenosine triphosphate (ATP) molecules into discrete steps along the filament (Howard, 2001). Two-headed motors walk 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 (Svoboda *et al*., 1993; Yildiz *et al*., 2003). Each step corresponds to a motor displacement of about 10 nm, comparable to the size of the motor heads. If there is no shortage of ATP, the motor makes about 100 steps in 1 s which leads to a velocity of about 1 μm/s. Cells use motor driven transports along two families of cytoskeletal tracks. Actin filaments are used by myosins and microtubules are used by both dyneins and kinesins.

Directionality

Molecular motors move unidirectionally along actin filaments or microtubules, transporting cargo within the cell. Transport of vesicles and organelles along axonal microtubules occurs both towards (anterograde) and away from (retrograde) the axon tip, mediated by motors of opposite polarity.

Disruption of either plus- or minus-end directed movement in axons or during mitosis completely disrupts the process, indicating that movement of opposite polarity is essential to carry out these basic cellular processes. Kinesin motors carry out both plus- and minus-end transport in the cell (Endow, 1999).

In biological cells, the motion of cargo particles along microtubules is bi-directional in the sense that the particle frequently switches its direction of motion. Since both kinesin and dynein motors are bound to these particles, the bi-directional motion arises from the competition between these two motor species (Lipowsky *et al.*, 2006)

Another important property that helps in the directed motion is processivity, i.e. the ability to take many steps along the microtubule before detaching from it. In the case of dimeric kinesin motors, binding of the second head to the next site along the microtubule takes place before the first head detaches. It results in a transient 'two heads-bound' state that permits long runs before motor detachment.

On large length and time scales which exceed a few microns and a few seconds, respectively, molecular motors perform peculiar motor walks. These motor walks consist of alternating sequences of active directed movements along filaments and passive non-directed diffusion. A molecular motor performs active directed movement along

a filament and unbinds from it after a certain walking distance. The unbound motor diffuses passively in the surrounding fluid until it rebinds to the filament and resumes directed motion (Lipowsky *et al.*, 2001).

Intracellular trafficking of pathogens and motors

Intracellular movement of pathogens is an active process and involves either the actin or the microtubules cytoskeleton. Propulsion by actin polymerization is used by cytosolic bacterial (*Listeria, Shigella, Rickettsia*) and viral (Vaccinia) pathogens (Gouin *et al.*, 2005). Microtubules and associated molecular motors are important for the cytoplasmic transport and also for the establishment and the stability of the bacterial replicative niche (Henry *et al.*, 2006). Viruses use the host actin and microtubule transport systems and their motors for several steps during their life cycle. Viruses induce rearrangements of cytoskeletal filaments, so that they can utilize them as tracks or shove them aside when they represent barriers.

In the cell periphery and in the nucleus, transport is mediated by the actin system, either by newly polymerized actin filaments pushing a particle, or by myosins moving along actin filaments. The motor proteins cytoplasmic dynein and kinesin catalyse transport along microtubules, thus bridging the gap between the periphery and the cell centre.

Virus interactions with the cytoskeleton differ between virus families, and no virus is currently known to use all of the mechanisms, but might employ different strategies. The hypothetical virus binds to the plasma membrane and surfs along filopodia towards an area of high endocytic activity, where it is internalized by endocytosis. Alternatively, the virus can fuse with the plasma membrane. After traversing the actin cortex either inside endocytic vesicles or by itself, free viruses or viruses inside vesicles are transported by dynein/dynactin along microtubules towards the MTOC. From the MTOC, viruses are transported towards the nucleus, possibly aided by the nuclear import/export machinery. Upon binding to the nuclear pore, the virus releases its genome for replication.

Biological motors and nanotechnology

Nanotechnology is enabling the miniaturization and fabrication of devices in a scale ranging from one to a hundred nanometers. A nanomachine is the most basic functional unit consisting of nano-scale components and able to perform a specific task at nano level, such as computing, data storing, sensing and actuation. Nanomachines are developed using individual molecules as building blocks (Bottom up), by downscaling current existing microscale device components (Top down) or using biological nanomachines found in nature as models (Biohybrid). Biological motor molecules possess many of the characteristics required to power nanomachines, such as high efficiency, small size required to operate in a highly parallel manner and can be modified through genetic engineering besides their easy production. A wide array of biochemical tools has been developed to manipulate these proteins outside the cell, which include microrotary motor driven by the gliding bacterium *Mycoplasma mobile* (Hiratsuka *et al.*, 2006), smart dust bio sensor (Bachand *et al.*, 2009), nanoactuator (http://www.medgadget.com), nanochopper and nanorobot.

Limitations of biological motor based nanodevices

The major limitation is inherent instability and restrictions in the environmental conditions they operate in (Browne & Feringa, 2006). A major difficulty in operating molecular machines lies not in achieving motion at the molecular level but in controlling their operation, especially their directionality. Molecules are in constant motion, if not frozen around 0 K, but their Brownian motion is random. Overcoming this randomizing effect and generating directional motion at the molecular level with artificial systems is a challenge (Mandl and Konig, 2004). To translate molecular movement to macroscopic levels, many molecular motors must be able to work cooperatively.

CONCLUSION

These biomolecular motors of different classes interact with each other directly or indirectly. It is still unknown how the nanomotors determine which cargo is to be transported and when to transport the cargo to its proper location within the cell. These biomolecular motors optimised for their efficiency by nature has served as a major source of inspiration for scientists to conceptualize, design and build — using a bottom-up approach — entirely synthetic molecular machines. Linear and rotary molecular motors have been anchored to surfaces without loss of function — a significant step towards future nanomachines and devices.

The possibility to exploit the structures and processes of biomolecules for novel functional materials, biosensors, bioelectronics and medical applications has created the rapidly growing field of nanobiotechnology. The recent explosion of research in nanotechnology, combined with important advances in molecular biology has created a new interest in biomolecular machines. The first goal in the biomolecular machine development is to use various biological elements as machine components that perform the same function in response to the same biological stimuli but in an artificial setting. Molecular motors are well-established nanoscale molecular machines present in living systems. They are responsible for various dynamical processes for transporting single molecules over small distances to cell movement and growth. Molecular motors are self-guiding and ideal systems because of their small size, perfect structure, smart and high efficiency. In general, active biomimetic systems based on molecular motors and filaments have many potential applications in bioengineering, pharmacology and medicine. Molecular motors could form the basis of bottom-up approaches for constructing active structuring and maintenance at the nanometer scale.

FUTURE PROSPECTS

These biomolecular motors can be used in diseases associated with impaired cargo transport, target for pharmacological therapy, for molecular repair of the human body and for delivery of genes or drugs to the

nucleus of cells or to the central nervous system. Biomolecular motor based devices can be used for environmental, biodefense or biomedical monitoring applications, and they provide miniaturized tools for diagnostic as well as therapeutic purposes. Molecular motors might also be applied as a drug delivery vehicle to the cell bodies of motor neurons by axonal transport as reported by Gunawardena *et al.* (2004). There are many challenges in applying protein nanomotors to nanotechnology. All these developments will be solely up to the imagination and skills of researchers, both in engineering and in natural sciences to envision the future and limitations of use for such highly intriguing devices that nature has designed and perfected over billions of years and is giving to us for our own use. Thus, automatic movement of these biomolecular motors is still under development and the field is under maturation.

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