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Molecular Motors A review article

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Abstract:

Molecular motors are essential agents of movements in living organisms, harnessing chemical free energy released by hydrolysis of ATP, belong to the same protein family, the P-loop NTPases.
In terms of energetic efficiency, they can be superior to man-made motors. They operate by small increments, converting changes in protein conformation into directed motion.

Key words: ATP, Dynein, Kinesin, Motor, Molecular Motor, Myosin, P-loop NTPases

Introduction:

Molecular motors are nanoscale biological devices that consume energy in one form and convert it into motion or mechanical work (Figure 1). They are essential agents of movement in living organism.

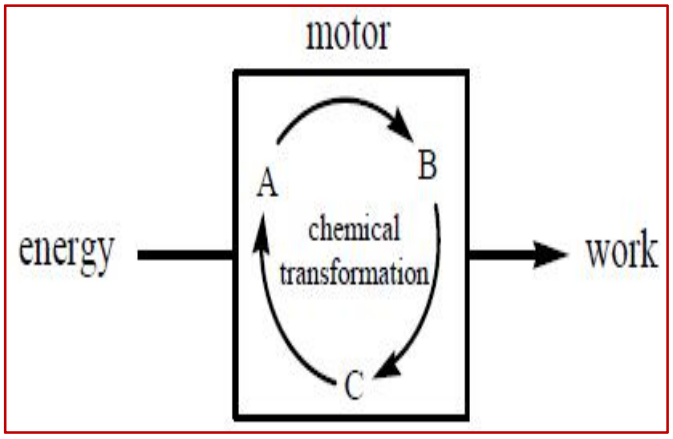


Figure 1: Schematic of a molecular motor.

Remarkably, the fundamental biochemical mechanisms that produce contractions in our muscles are the same as those that propel organelles along defined paths inside cells.

Many protein based molecular motors harness the chemical free energy released by the hydrolysis of ATP in order to perform mechanical work. In fact, many of them are members of the same protein family, the P-loop NTPases.

In terms of energetic efficiency, these types of motors can be superior to currently available man-made motors. One important difference between molecular motors and macroscopic motors is that, molecular motors operate in the thermal bath, an environment where, the fluctuations due to thermal noises are significant.[1,2,3,4,5]

General mechanism of operation [How do they operate]:

They operate by small increments, converting changes in protein conformation into directed motion. Orderly motion across distances requires a track that steers the motion of motor assembly. Such tracks can be DNA and RNA molecule or action and microtubule protein filaments.

The motor protein cycles between forms having high or low affinity for the track in response to ATP binding and hydrolysis, enabling a bind, pull and release mechanism that generates motion.

A completely different strategy for generating motion is used by some bacteria, such as E. coli. Here, a set of flagella act as propellers, rotated by a motor in bacterial cell membrane. This rotary motor is driven by a proton gradient across the membrane, rather than by ATP hydrolysis. The mechanism for coupling the proton gradient to rotary motion is analogous to that used by the F_0 subunit of ATP synthase.

Thus, both the major modes of storing biochemical energy – namely ATP and ion gradients have been harnessed by evolution to drive organized molecular motion.[1,2,4,6,7,8]

Classification and Examples:

Some of the molecular motors use ATP as energy source e.g. Myosins, Kinesins, Dyneins etc. (see figure 4 and 6b).

Some use GTP as energy source e.g. Dynamin which helps in endocytosis.

Some use proton gradient for driving the rotor e.g. ATP synthase, bacterial flagella etc.

Some use DNA as tack e.g. Topoisomerase, DNA polymerase.

Some use RNA as track e.g. RNA polymerase.

Experimental observation:

The activity of molecular motors is observed with many different experimental approaches (see figure 2), such as

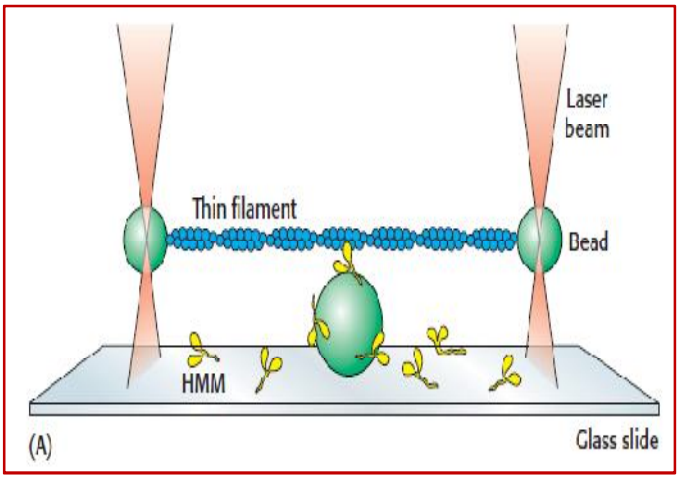


Figure 2: Single motor protein in action[1]

Fluorescent methods:

Fluorescence resonance energy transfer (FRET)

Fluorescence correlation spectroscopy (FCS)

Single molecule electrophysiology - Can be used to measure the dynamics of individual ion channels.

Optical tweezers – Are well suited for studying molecular motors because of their low spring constants.

Magnetic tweezers – Can also be useful for analysis of motors that operate on long pieces of DNA.

Many more techniques are also used. As new technologies and methods are developed, it is expected that knowledge of naturally occurring molecular motors will be helpful in constructing synthetic nanoscale motors.[4,8,10,11]

Eukaryotic Molecular Motors:

Eukaryotic cells contain 3 major families of motor proteins – myosins, kinesins and dyneins.

1) Myosin:

Derived from the Greek word for muscle, myosin is the protein responsible for generating muscle contraction. Human genome appears to encode more than 40 distinct myosins; some function in muscle contraction and others participate in variety of other processes such as cell division, pinocytosis, and hearing. In plants and fungi, myosin is also involved in cytoplasmic streaming, wherein cytoskeletal networks are formed in the cell, which allow cytoplasm to stream in a particular direction.

Structure:

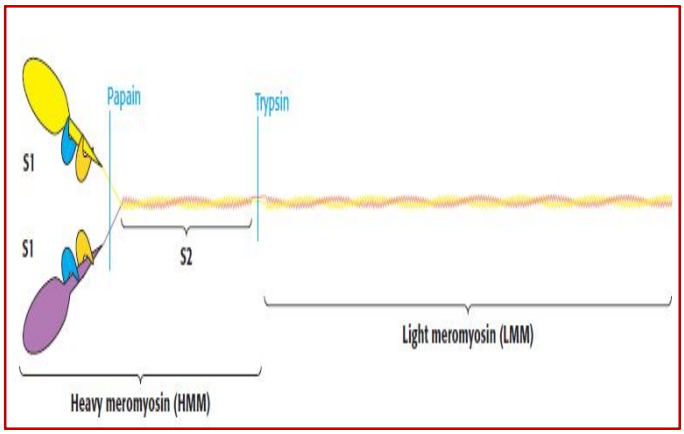


Figure 3: Myosin[1]

A motor protein consists of an ATPase core and an extended structure. Electron microscopic studies of skeletal muscle myosin show it to be a two headed structure linked to a long stalk.

The myosin molecule consists of 6 polypeptide chains, 2 heavy chains each with a molecular weight of about 200000 and 4 light chains with molecular weight of about 20000 each.

The 2 heavy chains wrap spirally around each other to form a double helix. One end of each of these chains is folded into a globular polypeptide structure called the myosin head. In the core of this head is the site for ATP binding and hydrolysis. There are two free heads lying side by side at one end of the double helix myosin molecule, the elongated portion of the coiled helix is called the tail.

The four light chains are also part of the myosin heads, two to each head. These light chains help control the function of the head during muscle contraction.

This is the structure of myosin II present in skeletal muscle. Other types of myosin are similar in structure, only they differ in the tail end where the amino acid sequences are different and they may have only one head instead of two. The assembly and functional properties of nonmuscle myosin are controlled by phosphorylation of serine and threonine residues in their tails. The tail end bind to the 'cargo' they carry while the head walks along the actin filament.

Function:

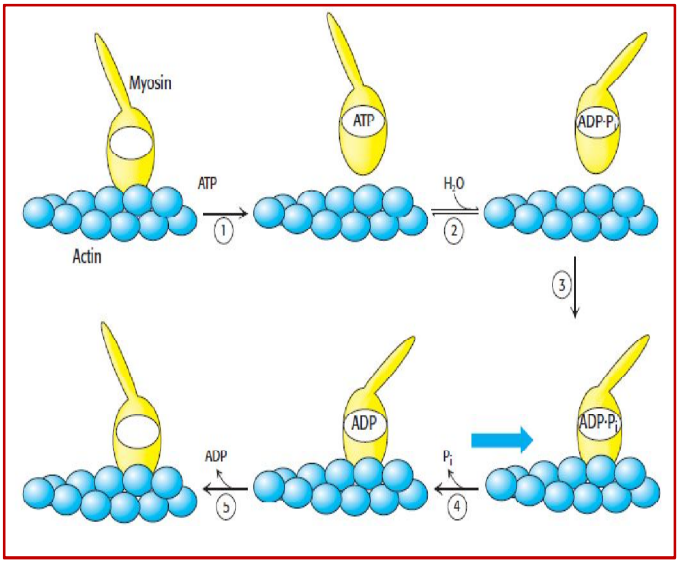


Figure 4: Myosin motion along actin[1]

We know the process of muscle contraction. How does ATP hydrolysis drive the power stoke? A key observation is that the addition of ATP to a complex of myosin and actin results in the dissociation of the complex. Thus, ATP binding and hydrolysis cannot be directly responsible for the power stroke.

Let us begin with myosin-ADP bound to actin. The release of ADP and binding of ATP to actin results in the dissociation of myosin from actin.

The binding of ATP to the myosin head, leads to a conformational change allows the relay helix to

adjust its position (A long helix of heavy chains that binds the light chains and protrudes outward from the head domain is known as 'lever arm'. A long α helix that connects the lever arm to the head, is known as the relay helix). The carboxyl terminal end of the relay helix interacts with structures at the base of the lever arm and so a change in the position of relay helix leads to a reorientation of the lever arm.

This results in movement of myosin head along the actin filament by approx. 110 Å. The ATP in the myosin head is then hydrolyzed to ADP & Pi, which remain bound to myosin. The myosin head can then bind to the surface of actin, resulting in the dissociation of Pi from myosin. Phosphate release in turn leads to a conformational change that increases the affinity of the myosin head for actin and allows the lever arm to move back to its initial position. The conformational change associated with phosphate release corresponds to the power stroke. After release of Pi, the myosin remains tightly bound to the actin and the cycle can begin again.

In the muscle, each head cycle approximately 5 times per second with a movement of 110 Å per cycle. However, because hundreds of heads are interacting with the same actin filament, the overall rate of movement of myosin relative to actin filament may reach 80000 Å per second, allowing a sarcomere to contract from its fully relaxed to its fully contracted form rapidly. The force generated is about 5 picoNewtons.

One molecular motor from bacteriophage Φ 29 (phi 29) can generate force upto 57 to 60 picoNewtons. That is an enormous force sufficient to lift 6 aircraft carriers. For comparison, the molecular motor RNA polymerase exerts a maximum force of 15 to 20 picoNewtons. A similar motor DNA polymerase exerts a force of 35 picoNewtons.

Role of length of lever arm:

A key feature of myosin motors is the role of the lever arm as an amplifier. The lever arm amplifies small structural changes at the nucleotide binding site to achieve the 110 Å movement along the actin filament that takes place in each ATP hydrolysis cycle.[1,6,8,12,13]

Applied:

1) In the Usher syndrome, the genes encoding myosin VIIa is mutated. This produces splayed stereocilia of hair cells of cochlea which do not function well, producing deafness.
2) Mutations of myosin Ib which is part of the adaptation motor that adjust the tension on stereocilia of the hair cells causes deafness.
3) Similarly mutations of myosin VI cause deafness.

2) Kinesin:

The conventional form of kinesin is a double head molecule that moves its cargo towards the positive end of the microtubules. Thus it is a positive end directed molecular motor producing antegrade transport i.e. it moves its cargo away from the center of the cell.

Some kinesins are negative end directed motors producing retrograde transport. Human genome encodes more than 40 kinesins.

Structure –

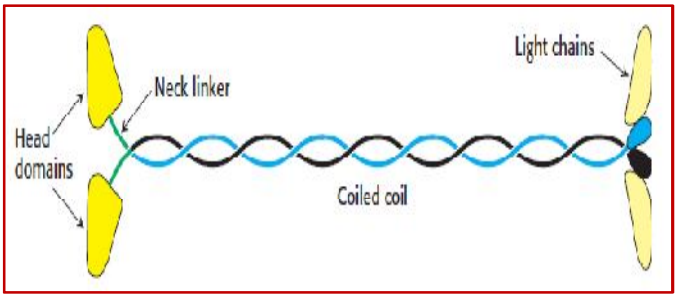


Figure 5: Kinesin[1]

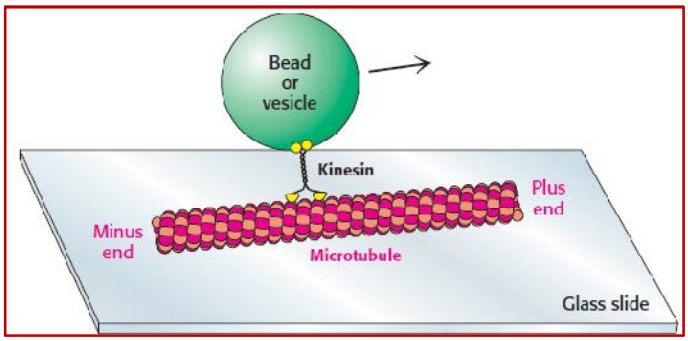
It has several features similar to myosin. The dimeric protein has two heads, linked by an extended structure. The size of head domain is

approximately 1/3 of that of myosin. The heavy chain is ½ the size of that for myosin.

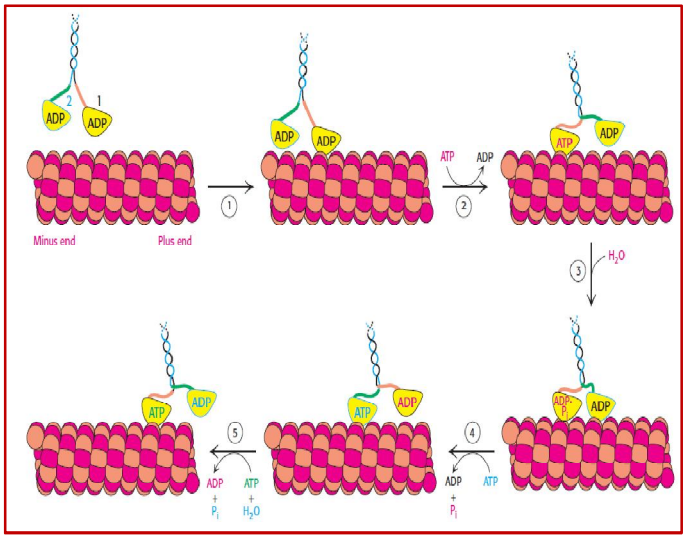
The head is built around a P-loop NTPase core. A region of approximately 500 amino acid follows the head domain. It forms an α helical coiled coil. Unlike myosin, the α helical region directly adjacent to the head domain is not the binding site for kinesin light chains. Instead, kinesin light chains, if present, bind near the carboxyl terminus.

Kinesins have a relay helix similar to myosin that can adopt different configurations when kinesin binds different nucleotides. It lacks an α helical lever arm. Instead, a relatively short segment termed 'the neck linker' changes conformation in response to nucleotide binding.

Function –



(a)



(b)

Figure 6 (a) and (b): Kinesin moving along a microtubule[1]

When a kinesin molecule moves along a microtubule, the two head groups of kinesin molecule operate in tandem – one binds and then the next one does. A kinesin molecule may take several steps before both heads groups are dissociated at the same time. In other words the motion of kinesin is highly processive.

A single kinesin molecule will typically take 100 or more steps toward the positive end of a microtubule in a period of seconds before the molecule becomes detached from the microtubule. Average step size is approximately 80 Å.

Addition of ATP strongly increases the affinity of kinesin for microtubules. This is in contrast with myosin. This does not mean that kinesin and myosin operate by completely different mechanisms.

Kinesin generated movement appears to proceed by a mechanism that is quite similar to that used by myosin. Let us begin with a two head kinesin molecule in its ADP form, dissociated from a microtubule. The neck linker binds the head domain when ATP is bound and is released when ADP is bound. The initial interaction of one of the head domains with a tubulin dimer on a microtubule stimulates the release of ADP from this head domain and the subsequent binding of ATP. The binding of ATP triggers a conformational change in the head domain that leads to two important events. First, the affinity of head domain for the microtubule increases, essentially locking this head domain in place. Second, the neck linker binds to the head domain. This change repositions the other head domain acting through the coiled coil domain that connects the two kinesin monomers.

In its new position, the second head domain is close to a second tubulin dimer, 80 Å along the microtubule in the direction of the positive end. Meanwhile, the intrinsic ATPase activity of the first head domain hydrolyzes the ATP to ADP and Pi.

When the 2nd head domain binds to the microtubule, the 1sthead releases ADP and binds ATP.

Again, ATP binding favors a conformation change that pulls the 1st domain forward. This process can continue for many cycles until by chance, both head domains are in the ADP form simultaneously and kinesin dissociates from microtubule. Because of the relative rates of component reactions, a simultaneous dissociation occurs approximately every 100 cycles.

Kinesin hydrolyzes ATP at a rate of approximately 80 moles per second. Thus, given the step size of 80 Å per ATP, kinesin moves along microtubule at a speed of 6400 Å per second.

Proposed mechanism:

Kinesin accomplishes transport by essentially 'walking' along a microtubule. Two mechanisms were proposed to explain how this movement occurs,

1) "Hand-over hand" mechanism – Kinesin heads step over one another, alternating the lead position. This is the mechanism described above.
2) "Inchworm" mechanism – One kinesin head always leads, moving forward a step before the trailing head catches up.

Despite some remaining controversy, mounting evidence points towards the 'hand-over hand' mechanism as being more likely.

Small structural changes can reverse motor polarity:

A small number of kinesins, including the protein ncd (for nonclaretdisjunctional, first identified in Drosophila), move towards the negative end. From an engineering perspective, there are many ways to change the polarity of a motor. How is the polarity changed in this case?

The mechanical parts of the motor domain including, the structures of the switch regions, the relay helix and the parts that bind microtubule are similar in conventional kinesin and the protein ncd.

Significantly however, the motor domain of protein ncd lies near the carboxyl terminus of the protein, whereas it lies near amino terminus in conventional kinesin. Furthermore, a short region just before the motor domain forms an α helix that docks against the motor domain in a position similar to that occupied by neck linker of conventional kinesin in the ATP form.

ATP binding by conventional kinesin leads to the binding of the neck linker, ATP binding by ncd releases the helical region. Its release allows the 2nd motor domain of the ncd dimer to bind to a site on the microtubule farther toward the negative end.

This shows us the economical refinement of a protein by evolution – in this case, subtle adjustments have produced an opposite mechanical result in the activity of the protein assembly.

Asters and assembly:

It has been found that microtubule based molecular motors, including a number of kinesins have a role in mitosis. The mechanism by which the cytoskeleton of the daughter cells separates from that of the mother cell was unclear. It seems that motors organize the two separate microtubule asters into a metastable structure independent of any external positional cues. This self-organization is inturn dependent on the directionality of the motors as well as their processivity (ability to walk).

Thus motors are essential for the formation of the mitotic spindle assemblies that perform chromosome separation. Specifically proteins from kinesin 13 family act as regulators of microtubule dynamics.

Applied:

Mutation in kinesin called KIF1B$_\beta$ causes most common peripheral neuropathy chracot-Marie-Tooth disease. Here a glutamine to leucine mutation

has been observed in the P-loop of the motor domain.

Mutations of kinesins involved in axoplasmic transport predispose to Schizophrenia.[1,2,4,5,8,13,14]

3) Dynein:

Dyneins are "minus end directed" motors as they transport various cellular cargos by 'walking' along cytoskeletal microtubules towards the negative end of the microtubule which is usually oriented towards the cell center.

Dyneins can be divided into two groups

1) Cytoplasmic dyneins
2) Axonemaldyneins also called ciliary or flagellardyneins.

Functions –

Axonemaldyneins cause sliding of microtubule in the axonemes of cilia and flagella.

Cytoplasmic dyneins performs functions necessary for cell survival such as organelle transport and centrosome assembly. It helps to position Golgi complex and other organelles in the cell. It also helps transport cargo needed for cell functioning such as vesicle made by the endoplasmic reticulum, endosomes, and lysosomes. It is also probably involved in the movement of chromosomes and positioning the mitotic spindles for cell division. It carries organelles and microtubule fragments along the axons of neurons in a process called axoplasmic transport. It also carries HIV to the nuclei of cells that have been infected.

Structure –

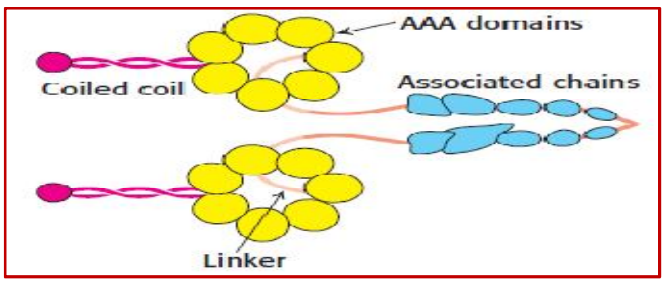


Figure7: Dynein[1]

Each dynein motor is a complex protein composed of many smaller poly peptide subunits.

i) Cytoplasmic dynein:

It has a molecular mass about 1500kDa, has approximately 12 poly peptide subunits – two identical heavy chains [520kDa] which has ATPase activity. Two 74kDa intermediate chains which anchor the dynein to its cargo. Four intermediate chains 53-59 kDa. Several light chains which are less well understood.

The force generating ATPase activity of each dynein heavy chain is located in its large doughnut shaped "head", two projections from the head connect it to other cytoplasmic structures.

One projection, the coiled coil stalk, binds to and 'walks' along the surface of the microtubule via a repeated cycle of detachment and reattachment. The other projection, the extended tail (also called 'stem'), binds to the intermediate and light chain subunits which attach the dynein to its cargo.

In eukaryotes, cytoplasmic dynein must be activated by binding of dynactin, another multisubunit protein that is essential for mitosis. Dynactin may regulate the activity of dynein, and possibly facilitates the attachment of dynein to its cargo.

ii) Axonemal dynein:

It comes in multiple forms that contain 1, 2 or 3 non-identical heavy chains (depending upon the organism and location in the cilium). Each heavy chain has a globular motor domain with a doughnut-shaped structure.

A coiled coil stalk that binds to the microtubule and an extended tail (or stem) that attaches to a neighboring microtubule of the same axoneme.

Each dynein molecule thus forms a "cross bridge" between two adjacent microtubule of the ciliary axoneme.

Applied:

Mutations of dyneins cause Kartagener's syndrome, immotile cilia syndrome.[1,4,8,10,15]

4) Synthetic molecular motors:

They are nanoscale devices capable of rotation under energy input. The prospect of synthetic molecular motors was first raised by the nanotechnology pioneer Richard Feynman in 1959.

The basic requirements of synthetic molecular motor are repetitive 360^0 motion, consumption of energy, unidirectional rotation.

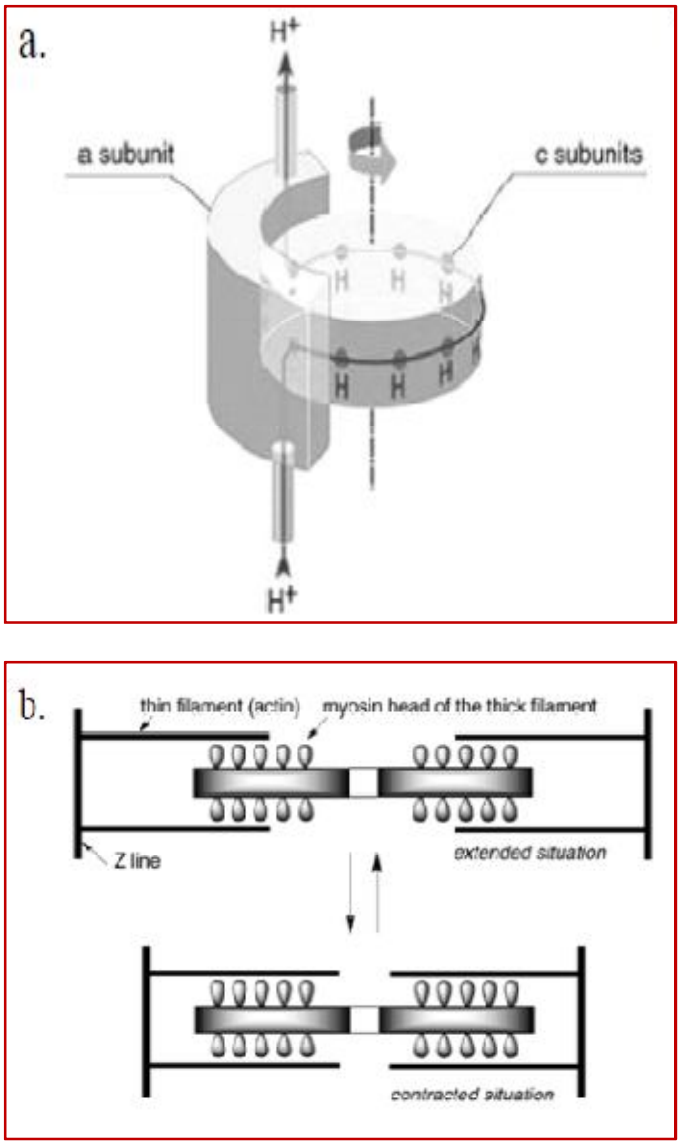


Figure 7: (a) The rotation of the Fo domain of ATP synthase is driven by a proton gradient. (b) The linear motion of muscle is driven by chemical energy as ATP is hydrolyzed.[15]

Examples:

i) Triptycene motors –

A 3 bladed triptycene rotor is connected to a rigid helicene scaffold. It is able to rotate 120^0 in a 5 step rotation sequence.

ii) Helicene motors –

It has monodirectional molecular rotor that rotates 360^0. The motor system consists of a bis-helicene connected by an alkene double bond. One cycle of unidirectional rotation takes four reaction steps.

iii) Nanocar –

The car thus far synthesized has an helicene derived engine with an oligo (phenyleneethynylene) chasis and four carborane wheels and is expected to be able to move on a solid surface with atomic force microscopy monitoring.[3,15]

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