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Review article

Nature's design of nanomotors☆

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Abstract

The need for movement is an essential concept of all living organisms. On a macroscopic scale, animals and microbes have to be able to move towards food and away from poison and predators. Plants turn their leaves toward their energy source, the sunlight. But even on a molecular scale, movement is essential for life. It has been known for a long time that enzymes and proteins undergo large conformational changes while performing their biological tasks. The catalytically active regions of enzymes need to sequentially open to bind their respective substrates and close to allow the specific chemical reaction to occur in a defined chemical environment. The active sites finally open up again up to allow the product to be released. Molecular motors are proteins and protein complexes that have evolved in living cells to carry out a variety of functions essential for survival, reproduction and differentiation of the cells and organisms. They use chemical, electrochemical or potential energy and transduce that energy into physical, chemical or mechanical force. In this paper we review some of the molecular motors that were designed by nature to either perform physical work or that contain motor-like movements as part of their catalytic mechanism.

Keywords: Molecular motors; Rotary motors; Linear motors; ATPase; Dynein; Kinesin; Myosin

1. Introduction

Most molecular motor proteins perform actual physical work by actively transporting or moving other molecules or proteins within cellular systems. Examples of these type of proteins are the kinesin motor proteins that 'walk' on 'tubulin-rails' transporting vesicles along given pathways in the cell. Similarly, certain members of the myosin family transport cargo using actin filaments as a track to run on. In addition to intra-cellular transport directed by microtubule tracks, proteins like dynein together with tubulin are also involved in the movement of whole, free cells like sperm cells and protozoa.

The concerted sliding of actin protein filaments on myosin motor-filaments causes the contraction of whole sub-cellular organizations in muscle cells during muscle contraction or allows migration and division of cells. Most of these systems utilize chemical energy in form of adenosine-5′-triphosphate, ATP, to power the respective conformational transitions.

Bacteria use the energy stored in proton gradients that are generated across a phospholipid membrane to drive the rotary movement of flagella, elongated structures that enable bacteria to 'swim' to their source of nutrition. This molecular machine should actually be named as an example of a 'micro motor' due to the size and complexity of the flagellar motor protein complex.

The molecular machine qualifying as Nature's smallest rotary motor is a protein complex whose physiological function is not that of a physical motor. This specific enzyme was designed to synthesize adenosine-5′-triphosphate, ATP, Nature's universal energy currency, by using the energy inherent in food sources or in sunlight (depending on the origin of the enzyme). This enzyme is called the F_0F_1-ATP synthase and it is, like ATP itself, ubiquitous in all organisms.

The synthase has intrigued researchers for more than four decades for a variety of different reasons. In a paper entitled 'The ATP Synthase—A Splendid Molecular Machine',

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published in 1997 [1], Paul D. Boyer from the Molecular Biology Institute at UCLA who shared the 1997 Nobel Prize for Chemistry with the British scientist John Walker for their accomplishments concerning their research on the ATP synthase, stated that "All enzymes are beautiful, but the ATP synthase is one of the most beautiful as well as the most unusual and important". (The third person sharing this 1997 Nobel Prize for Chemistry was Jens Skou for his work on P-type ATPases).

All in all, coordinated movement is a concept that nature has used and perfected for billions of years. Only within these last few years, when humans have started to design smaller and smaller artificial devices, reaching into the nanometer scale, have these cellular and sub-cellular natural organizations been termed with names like 'Nanomotors' or 'Nanomachines'. However, the design of these motor proteins is intriguing and it is fascinating to envision the potential use of biological devices within nanomechanical arrangements.

After describing the 'physical' motor proteins kinesin, dynein and myosin, this paper will discuss how the ATP synthase accomplishes its natural goal, the synthesis of ATP, while also being Nature's smallest and most efficient molecular motor.

2. Kinesin, dynein and myosin: motors for linear, intracellular transport

Internal order in eukaryotic cells is created by using protein motors that shuttle organelles and molecules along cytoskeletal tracks consisting of self-assembling proteins like tubulin and actin.

The three known families of cytoskeletal motor proteins kinesin, dynein and myosin share a variety of important features. All family members are multi-protein complexes and are able to achieve movement by binding and hydrolyzing ATP in a globular motor domain of their so-called heavy chains. The motor domain is chemically and functionally coupled to an extended tail that mediates binding of the cargo to the motors. The interaction between motor and track is mediated by the motor domains and is highly precise. Each motor specifically binds to a particular filament and is actively moving cargo only in one direction of the polarized track-filaments. Proper trafficking of macromolecules within cells depends on these highly organized cytoskeletal filaments and the directionality of the motors they are associated with. The systems are self-guiding systems and highly efficient. They can travel at high velocities for rather long distances before dissociating from the respective track.

Recent developments in elucidation the molecular structure of these motors as well as advances in the techniques used to investigate their mechanisms have brought forward a wealth of information that led us to a better understanding of the similarities and differences of these linear nanomotor systems.

2.1. Kinesin

Kinesin is a motor protein associated with microtubules and was identified in 1985 [2] as the force that underlies the movement of particles along microtubules within the giant axon in squid [3,4]. Conventional kinesin is a tetrameric protein that consists of two heavy chains, each of which contains a coiled coil dimerization domain and a tail. The cargo-binding region resides on two light chains associated with the heavy chains [5,6]. A simplified cartoon of kinesin is shown in Fig. 1, top. In the figure the motor domain and tubulin-binding domain is symbolized as an oblong structure and is pointing to the left. The cylindrical shapes at the right of the figure symbolize the cargo-binding domain. The motor domain binds the fuel, adenosine-5′-triphosphate, ATP, and hydrolyzes it into the corresponding adenosine-5′-diphosphate and inorganic phosphate. In a concerted action the two motor domains of the dimer use the binding energy of ATP as well as the chemical energy stored in the β-γ-anhydride bond of ATP to trigger conformational changes within a hinge region (that connects the tail to the motor domain) to move the motor domains in a hand-over-hand movement along

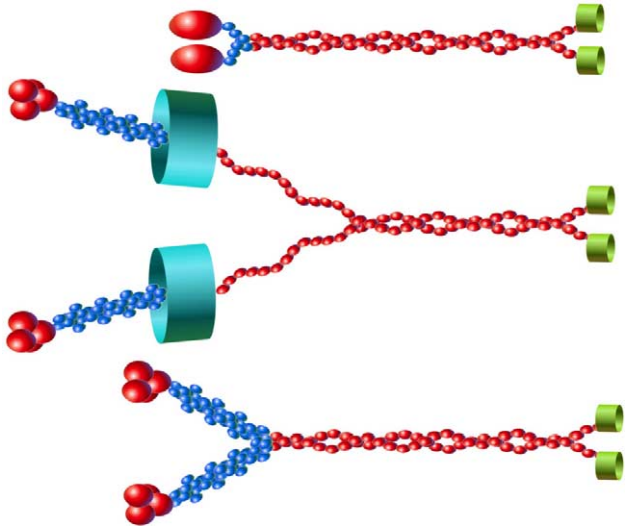

Fig. 1. Different types of molecular motors: top, cartoon of kinesin: the globular domains to the left are the motor domains that bind and hydrolyze ATP and that attach and move along the tracks. The coiled coil structure of the tail couples the cargo-binding domain, depicted as a ring, to the motor domain. Second from top, cartoon of dynein: the stalks extending from the ring structures to the left and that depict the motor domains bind to the microtubules with their globular tip regions. Cargo attachment is at the opposite end of the tail (to the right in the figure), also depicted as a ring structure. Third from top: cartoon of myosin V. The globular domains at the left are the motor domains that also interact with the track substrate, here the actin filaments. The longer 'legs' consist of calmodulin and end in a coiled coil tail that ends in the cargo-binding domain, depicted as a ring structure at the right. All of the motor proteins are significantly simplified for these cartoons. Each of the protein in addition contains a multitude of helper proteins that allow function and specify the cargo.

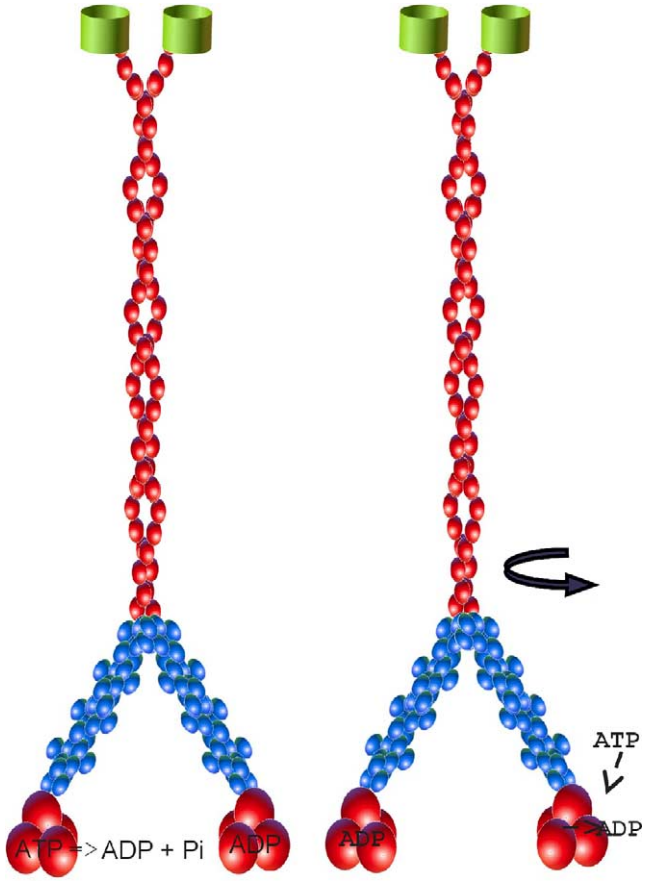

Fig. 2. Schematic of linear movement of motor proteins like kinesin or non-muscle myosin: the motor domains bind to the corresponding tubulin or actin filaments upon binding of nucleotides. ATP-hydrolysis in one of the motor domains primes the motor for movement. Movement is then accomplished by exchange of ATP for bound ADP in the second motor domain and a rotation of the back 'hand' into the front. Thereby a step-by-step or hand-over-hand movement is accomplished.

the tubulin rail. A schematic model on how ATP-hydrolysis is converted into mechanical movement is shown in Fig. 2. ATP hydrolysis in one of the 'hands' primes the protein for motion. After exchange of bound ATP for ADP in the second motor domain, a conformational change occurs that allows the back 'hand' to swing over and perform a step of distinct distance. This action of movement is very similar also for the big brother of kinesin, myosin V, that will be described in Section 2.3. The direction of movement is towards the fast polymerizing/depolymerizing plus end of the microtubules [7].

2.2. Dynein

The second microtubule associated motor protein that was for the longest time thought to be singly responsible for the reverse, retrograde axonal transport is dynein, transporting cargo towards the minus end of the microtubules [8]. Most of the about 15 forms of dynein found in vertebrates are involved in ciliary and flagellar movement. Only two members of the family are cytoplasmic, one of which, cytoplasmic dynein 1, formerly called MAP1C (microtubule associated protein 1C), is responsible for a wide range of cellular functions. A cartoon of dynein is shown in Fig. 1, second from the top. Again, the tubulin-binding domains are pointing to the left, the motor domains are symbolized by the large cylindrical structures towards the left of the figure. The cylindrical shapes on the right symbolize the cargo-binding domain. The functions associated with cytoplasmic dynein include several aspects of chromosome behavior, mitotic spindle orientation, nuclear and cell migration as well as the cellular transport of a variety of vesicles. The second member of the cytosolic dyneins, dynein 2, is much more restricted in its functions. In cell cultures of mammalian cells it is seen associated with cilia and in the region of the Golgi apparatus [9,10]. In tissues it seems limited to ciliated cells and to ciliary structures [10].

All forms of dynein consist of a dimer of heavy chains that contain the motor domain as well as a variety of accessory proteins termed intermediate, light intermediate and light chains. Some of these accessory proteins are common for both cytoplasmic and axonemal dyneins, others are specific to the subclasses. In contrast to both kinesin and myosin, the dynein heavy chain is very large with a molecular weight >500 kDa. The heavy chain folds into a ring-like structure composed of six ATP binding domains as well as a stem and a stalk that emerges from the head domain (for review see [11]). The stalk takes the structure of a coiled coil and contains the microtubule-binding domain [12,13] (for review see [14]). A swing of the stem and stalk emerging from each of the head-domains that is induced by ATP-hydrolysis was proposed to be responsible for the power-stroke that allows movement along the microtubules [15]. Very recent work showed that a recombinant, single headed C-terminal fragment of dynein was able to drive minus-ended sliding of microtubules, indicating that each of the motor-protein complexes can work independently [16].

2.3. Myosin

The third family of linear motor protein to be discussed here are the myosins. This superfamily of proteins powers the directed movement of cargo on actin filaments in contrast to the previously discussed kinesins and dyneins that move on tubulin filaments or microtubules. They function in a wide variety of cellular tasks, from cellular transport to muscle contraction (for recent reviews see [17,18]). The best studied and longest known members of the myosin family are the myosins I and II, which in complex with actin filaments (thick filaments) are responsible for muscle contraction in cardiac and skeletal muscle. To achieve this function, myosin polymers (thick filaments) bind to actin (thin) filaments in an ATP-dependent fashion. In a concerted action of the polarized heavy filaments they propel the thin filaments together, thereby shortening the muscle fibers and contracting the muscle. Similar to kinesin, myosin consists of a homodimer of heavy chains and a tail

consisting of coiled coil light chains. The heavy chains contain the globular motor domains, where in an ATP-binding and hydrolysis dependent mechanism, conformational changes are generated that result in muscle contraction. In contrast to kinesin and dynein, muscle type myosins do not 'walk' along the actin filament, but bind the substrate (actin filament) tightly, perform a conformational change that pulls the filaments for a distance of 10 nm and then release the substrate. This process is performed multiple times until the desired muscle contraction is reached. Already almost 20 years ago, researchers showed that the myosin motors of skeletal muscle were also functional in vitro. Myosin immobilized onto a glass plate was able to move actin filaments at a velocity of about 3–4 μm per second in the presence of ATP [19].

The non-muscle members of the myosin family are involved in organelle transport, using actin filaments of the cytoskeleton as tracks, their mechanism of action is very similar to that described for kinesin and shown in Fig. 2. As an example, myosin V carries its intracellular cargo for long distances in a hand over hand fashion, very similar to kinesin. The movement is accomplished by taking multiple steps at a time before detaching from the actin substrate. Each step is powered by the hydrolysis of ATP [20–22]. The step size of myosin V was recently found to be 37 nm/ATP hydrolyzed [23] therefore qualifying as a 'big brother' to kinesin (top panel of Fig. 1) whose step size is 8 nm/ATP hydrolyzed [24]. A simplified cartoon of myosin V is shown in the third panel of Fig. 1. The globular domains to the left symbolize the motor domains; the cylinders pointing to the right are the cargo binding domains. In all cases, the cartoons of the motor proteins are simplified significantly and are missing various helper proteins that are necessary for proper function.

All of the discussed motor proteins have evolved to enable movement, either on cellular or supra-cellular levels and all of the motors consume chemical energy in form of ATP to perform their function.

The protein motor to be discussed next, the smallest rotary motor known, was not predestined by Nature to be used as a motor. Quite the contrary, it was brilliantly designed to produce the energy source, ATP, that is required for the movement of all the other motor proteins as well as a multitude of other cellular functions and biochemical pathways.

3. ATP synthase, structure and mechanism of a rotary motor

The F_0F_1-ATP synthase is found in all organisms and has intrigued scientists for almost four decades. This highly asymmetric protein assembly drives one of Nature's most challenging and probably the most important chemical reaction, namely the synthesis of adenosine-5′-triphosphate. The energetic challenge of this reaction is the formation of an anhydride bond between adenosine-5′-diphosphate (ADP) and inorganic phosphate (P_i) in an aqueous environment which would usually favor the hydrolysis and not the synthesis reaction. The energy for the synthesis reaction is provided by the flow of protons down an electrochemical gradient and across the corresponding energy coupling membrane, the plasma membrane in bacteria, the mitochondrial membrane in eukaryotes and the thylakoid membrane of chloroplasts in plants. In mitochondria and bacteria, the synthase efficiently couples the oxidation of $NADH/H^+$ to the formation of ATP. The reducing energy (in the form of two electrons) that is stored in $NADH/H^+$ is utilized in the respiratory chain, where the electrons are transferred in stepwise processes to oxygen that leads to the reduction of oxygen to form water. During these redox-processes protons (H^+) are pumped across the energy coupling membranes by a sequence of membrane proteins of the respiratory chain. As shown in Fig. 3, the energy stored in the generated proton-gradient drives the synthesis of ATP. Under certain in vivo conditions, e.g. in some bacteria when living in an anaerobic environment, the reactions of the synthase can be reversed so that ATP-hydrolysis-driven proton pumping occurs.

In photosynthetically active organisms, a light-driven proton translocation is coupled to the formation of

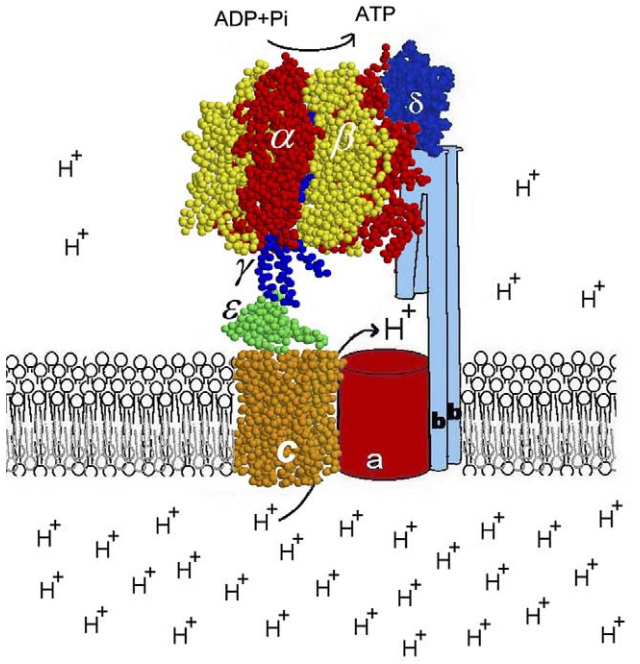

Fig. 3. Model of the F_0F_1-ATP synthase: the cartoon shows the two portions of the ATP synthase. The polypeptides *a*, *b* and *c* are partially or totally embedded in the membrane and constitute the F_0-portion. Subunit *a* and the ring of *c*-subunits are involved in the translocation of protons across the membrane. The membrane associated F_1 part consists of subunits α, β, γ, δ and ε and is responsible for the synthesis of ATP. The subunits $ab_2\alpha_3\beta_3\delta$ are considered to be the stator to the rotary motor. Subunits $c_{9-14}\gamma\varepsilon$ were shown in different experiments to rotate during catalysis. The X-ray structural model derived from [36] was used in this cartoon. The molecular coordinates are available in the protein data bank. The model was created using the program rasmol.

the terminal anhydride bond of ATP ($ADP + P_i \rightarrow ATP + H_2O$).

In vivo, under physiological conditions, the free energy that is stored in the terminal (β-γ)-anhydride bond of an ATP-molecule is about -9×10^{-20} J, equaling about −50 kJ per mol of ATP, depending on the specific cellular conditions.

The ATP synthases from different organisms are highly conserved. Much of the research on F_0F_1-ATP synthases has focused on the bacterial enzyme from *Escherichia coli*, mainly because molecular biological tools are easily available, but also because the *E. coli* enzyme is simpler in subunit composition and can be readily purified in rather large quantities. A second, favored source for purifying the ATP synthase or substructures thereof is the thermophilic bacterium PS3. In addition to easy availability, the PS3 enzyme is also very stable at ambient and elevated temperatures. Maximal catalysis rates are observed at temperatures of about 70 °C.

In all organisms the synthase consists of two components: the membrane embedded F_0-sector that is drives translocation of protons and the membrane associated part that contains the sites for nucleoside triphosphate synthesis and hydrolysis, the F_1-ATPase, see Fig. 2. Both parts of the enzyme are highly asymmetric. The F_0-part in the simplest ATP synthase from *E. coli* consists of three different polypeptides *a*, *b* and *c* with a stoichiometry of one *a*, two *b* and 9–12 copies of subunit *c* per functional unit [25]. Subunits *a*, *b* and *c* are either totally or partially membrane-embedded. Subunits *a* and *c* are directly involved in proton movement through the membrane (for review see [26]). Subunit *b* is anchored to the membrane with its N-terminal end. The residual amino acids of this protein project into the cytoplasm and interact partially with the F_1-ATPase part of the synthase thereby connecting it to the membrane.

The catalytic part of the synthase, F_1-ATPase, consists of the five subunits α through ε with three copies each of these largest α and β subunits and one copy each of the minor subunits, γ, δ and ε [27]. When removed from the membrane and uncoupled from the proton gradient, this F_1-part is capable of net ATP-hydrolysis only, due to the lack of energetic driving force provided by the proton gradient. This part of the enzyme is therefore named the F_1-ATPase.

Six nucleotide binding sites are located on the α and β subunits. The three binding sites located on the β-subunits are catalytically active and were therefore named catalytic sites. They show an extraordinary degree of positive catalytic cooperativity. When all of the three sites are filled with substrate, the rate of catalysis is at least 10^5 times faster than if only one substrate is bound to the catalytic sites, for more details see [28–30]. The function of the three sites located on the alpha subunits remains unclear and they are usually referred to as noncatalytic sites.

Already in the late 1970s, Paul D. Boyer presented a model for the reaction mechanism of the ATPase that was termed the 'Binding Change Mechanism' (reviewed in [31]). One of the tenets of the theory stated that every one of the catalytically active nucleotide binding sites of the ATPase is in a different structural and functional state at any given time during catalysis. During sequential catalytic cycles, all of the sites go through the same conformational and functional states. While one of the sites is empty of any substrates, ADP and P_i, a second site has both substrates bound and a third site is in a conformation that catalyzes the actual bond formation between ADP and P_i, resulting in elimination of water and the synthesis of ATP. The Binding Change Mechanism further states that the energy for the synthesis of ATP is solely required to release the product from the active site and not for the actual formation of the β-γ-anhydride bond. Earlier experiments had shown that the equilibrium between ADP + P_i and ATP in the catalytic site is close to unity. The idea that these sites sequentially participate in this mechanism suggested that binding of substrate at one site promotes the release of product at a second site. This proposed mechanism beautifully accommodated the data that had been gathered about structure, subunit composition and catalysis of the enzyme. One most important question that remained at this point was what type of conformational transition enables the catalytic sites to sequentially change their conformation and function. The first suggestion that rotation of internal subunits was part of the catalytic mechanism of the ATPase was published by Paul Boyer in the early 1980s (for review see [31]).

Tremendous progress in our knowledge about the mechanism of ATP hydrolysis and synthesis was made in this last decade, gaining even more momentum when a high resolution structural model of the beef heart mitochondrial F_1-ATPase was presented 10 years ago, in 1994 by John Walker's group [32]. An X-ray crystallographic model of the beef heart mitochondrial ATPase is shown in Fig. 4. The X-ray structure derived model gives detailed information about the structure of the nucleotide binding sites. In this model, two of the catalytic sites are rather similar in conformation, while the third catalytic site differs significantly from the others in structure. This third side does not have nucleotide bound (at least in the crystal structure), and a whole section of the binding site is rotated to form a very open conformation (able to release product). The model shows that all the noncatalytic sites are very similar in structure.

Several other structural models of various sub-assemblies of F_1 or F_0F_1 from a variety of different sources have become available over the last few years [33–37] increasing our knowledge about the structural basis of the ATPase and ATP synthase and allowing the design of experiments that drastically furthered our understanding of the molecular mechanism of the enzyme. For example, the structural models made rational mutagenesis of key-residues within the nucleotide binding sites possible, which allowed researchers to monitor the occupancy of the nucleotide sites during turnover by using biophysical techniques [38]. The data resulting from these experiments suggested that at

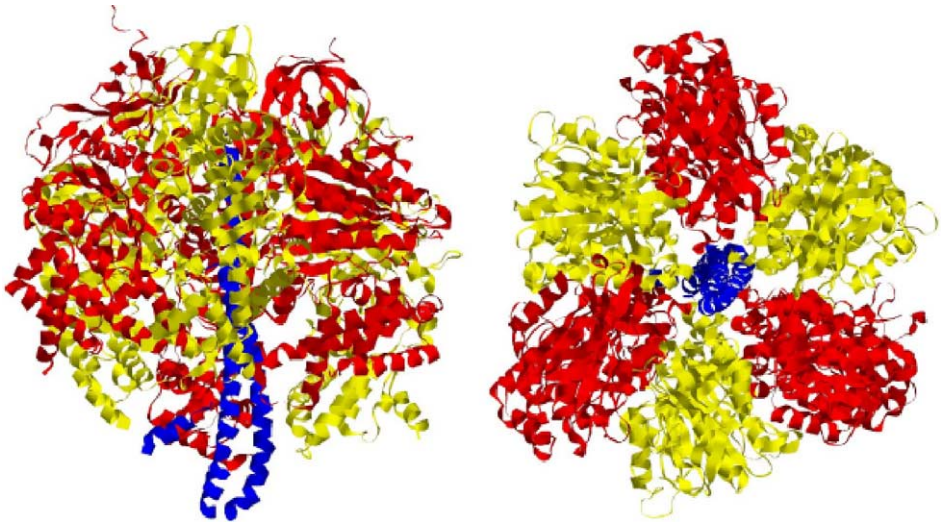

Fig. 4. X-ray structural model of the mitochondrial F_1-ATPase [34]. Left: side view, right: view from the membrane surface. The molecular coordinates of a variety of different F_1-ATPases from different sources are available in the protein data bank. The model was created using the program rasmol and the molecular coordinates from the protein data bank.

saturating ATP concentration and under maximal turnover conditions all three catalytic sites are occupied. The same results were obtained for isolated F_1 as well as for a solubilized F_0F_1-ATP synthase [38–42].

Experiments from our group using Electron Spin Resonance Spectroscopy and spin-labeled nucleotides also suggested that under equilibrium conditions almost all nucleotide-binding sites are filled [42–44].

3.1. Rotational mechanism of the F_1-ATPase

For the better of about 15 years, researchers tried to provide evidence that rotation of some of the subunits in F_1 was indeed a part of the mechanism of the ATPase. Especially after the X-ray structural models of the F_1 clearly placed the γ subunit into the central cavity inside the ring of three α and three β subunits and showed its asymmetric interactions with the $\alpha_3\beta_3$-ring, it became likely that γ may be the subunit rotating relative to $\alpha_3\beta_3$. In different chemical and biophysical approaches, two lab groups independently presented strong evidence that indeed such rotation was taking place [45–47]. It took, however, until 1997 for Yoshida's group to present their elegant experimental set-up that unequivocally and in real time showed that F_1-ATPase is indeed a molecular motor [48]. In Fig. 5 we present a cartoon of the experimental set-up that Noji et al. [48] used to show that rotation occurs. They used a sub-structure of the F_1-ATP synthase from the thermophilic bacterium PS3 that only contains the subunits α and β as well as the γ-subunit that resides in the middle of the $\alpha_3\beta_3$ ring. This assembly is very stable and efficient in ATP-hydrolysis. The β-subunits of the construct were genetically modified to each carry 6–10 histidine amino acid residues at one end of the β-polypeptide. These polyhistidines or 'His-tags', bind tightly to nitrilo triacetic acid that is chelated to Ni^{2+} ions via the carboxylic acid moieties and is commonly abbreviated as Ni-NTA. In the 'rotation' experiment, glass cover slips were covered with Ni-NTA and the His-tagged F_1 was added to the top of the cover slips which resulted in F_1 immobilized 'head down' onto the plates through its His-tags, meaning that the side of F_1 that is normally directed towards the membrane is pointed away from the glass plates in this set-up. The γ-subunit that protrudes from this 'upper' end (usually directed towards the membrane) of the $\alpha_3\beta_3\gamma$ substructure was also genetically modified to contain cysteine residues that can be reacted with sulfhydryl-specific reagents. In this case, the cysteines were modified with a reagent that introduced a biotin molecule to the γ-subunit of F_1. Biotin is a small organic molecule that very tightly and specifically binds to a protein called strepavidin. Streptavidin contains four binding sites for biotin.

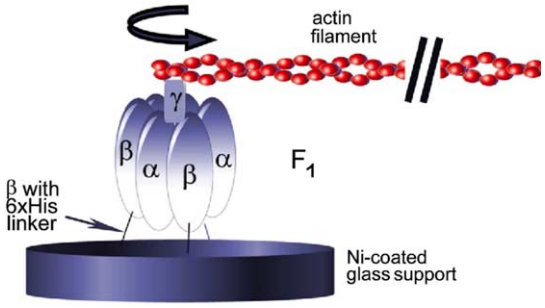

Fig. 5. Cartoon of a F_1-ATPase-actin construct as it was used to show rotation of the γ-subunit relative to $\alpha_3\beta_3$ [48]: a minimal structure of the F_1-ATPase from the thermophilic bacterium PS3 that consists of $\alpha_3\beta_3\gamma$ was used and was genetically engeneered to contain polyhistidine residues (His-tags) at the β-subunits. The His-tags were immobilized to glass cover slips that are covered with a Ni-NTA substrate. Using biotin–streptavidin–biotin interactions, actin filaments were attached to the F_1-motor. The actin filaments carried actin-specific fluorescent dyes. Rotation of the actin filament was observed in a fluorescence microscope.

After binding streptavidin to the biotin molecule that was attached to the γ-subunit, biotinylated actin filaments were added to the construct. Actin, as part of the contractile apparatus of the muscle and part of the cytoskeleton of individual cells, is a globular, monomeric protein that spontaneously polymerizes under physiological conditions to form stable elongated filaments. In the experiment described by Noji et al., actin monomers were first biotinylated (chemically reacted with biotin) and subsequently allowed to polymerize. They were in addition decorated with an actin-specific fluorescent dye, which made the formation of the filaments visible when excited by light of an appropriate wavelength that causes fluorescence to occur. Addition of these modified actin filaments to the immobilized, modified F_1-ATPase was the final step to visualize a very impressive molecular motor: in a fluorescence microscope, Noji and colleagues were able to observe the rotation of γ-subunit-associated actin filaments in real time.

In the initial experiments, they observed rotation of actin filaments of up to 4 μm length in the presence of ATP for several minutes and hundreds of revolutions. The rotation always occurred counter-clockwise when viewed from the direction of the membrane (or here from the top). Between 0.2 and 10 revolutions per second were observed depending on the length of the filaments attached (1–4 μm). The 'height' of the F_1-construct from glass plate to the end of subunit γ is about 15 nm, the length of the actin filament that was rotated by this motor was up to 4 μm, or 4000 nm, which makes it about 260 times the size of the F_1 molecular motor. To put this into a 'macroscopic' perspective, one needs to imagines F_1 to be the size of a human. If it were about 1.50 m tall, that motor would be able to rotate a pole that is about 500 m long and >1 m in diameter in an aqueous environment at rates of about one revolution per second.

Observing the rotation was not trivial in the beginning: thousands of assembled F_1-actin-filament constructs had to be monitored and rigorous controls were necessary in order to show without a doubt that ATP-hydrolysis-dependent rotation of the γ-subunit in fact took place. Considering the complicated set-up of the experiment and the dimensions of the reaction partners, this is certainly not surprising. A number of research groups, however, have since successfully repeated the experiments using F_1-ATPases from different sources and of different sub-structure composition as well as different attachments. Instead of the actin filaments also gold, polystyrene or magnetic beads were attached to γ and their rotation was observed.

In the meantime, various research groups were able to show that also the ring of *c*-subunits of F_0 rotates within the membrane, see Fig. 6 for cartoon of experimental set-up. When the driving force of the proton gradient across a membrane is strong enough, the downhill flow of the protons drives the rotation of the ring of *c*-subunits within the membrane, resulting in the rotation of the γ-subunit.

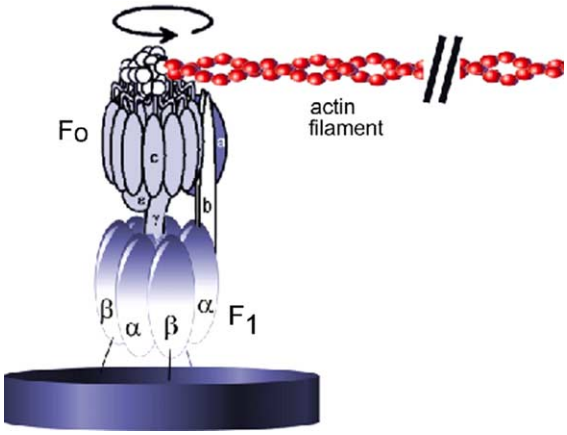

Fig. 6. Cartoon of a construct to show the rotation of the ring of subunits *c* [51]: in addition to the histidine-tag that allows the F_1-portion of the enzyme to be immobilized on glass slides, a second set of affinity tags (here the so-called Strep-tags) were introduced to the ring of subunits *c*. Strep-tags efficiently bind to streptavidin or related proteins, which in turn bind to the biotin-molecules that were attached chemically to the fluorescently labeled actin filament. The rotation of the actin filament again can be observed in a fluorescence microscope.

The rotation of the γ-subunit in itself appears to cause the necessary conformational change within the ATPase to allow ATP to be released. The process can also be driven in the opposite direction, where hydrolysis of ATP results in the rotation of subunit γ which then causes the rotation of the ring of subunits *c* resulting in the pumping of protons across the membrane [49–52] (for reviews see [53] and [54]).

In a recent paper [55] it was shown that when the rotor subunits *c* are immobilized onto Ni-NTA-coated glass plates using histidine tags on a Ni-NTA surface, those subunits of the synthase that usually do not participate in rotation (*a*, b_2 and $\alpha_3\beta_3$, usually referred to as the 'stator' subunits) rotated when ATP was added to an artificially prepared membrane system. The results indicated that the membrane embedded F_0F_1-ATP synthase can be divided into mechanical units that rotate relative to each other upon powering the system with fuel, ATP or a H^+-gradient.

3.2. Energy and torque considerations for the molecular motor F_1-ATPase

Isolated F_1-ATPase from different sources hydrolyzes ATP at rates between 100 and 500 molecules per second. The thermophilic enzyme that was used in these experiments hydrolyzes ATP at an average rate of 300 molecules ATP per second. This implies that a load-free motor rotates at 100 revolutions per second because three ATP are hydrolyzed per full rotation. The rotation of subunit γ was shown to be a three-stepped process [56–61], each step corresponding to the hydrolysis of one ATP molecule. At ATP concentrations below or at 1 μM, even smaller rotational sub-steps of 90 and 30° were resolved [60] that were attributed to the binding of ATP and the release of

ADP. Using the F_1-actin-filament construct with 1 μm filament length and low ATP concentrations that only allow slow rotation, Yasuda et al. [61] observed distinct steps where the actin filament rested in 120° intervals.

Calculated from the rotational velocity, the torque reached during rotation is >40 pN nm. By comparison, the sliding force that motors like myosin and kinesin generate is much smaller. The muscle type myosins produce a sliding force of 3–6 pN [62,63] and kinesin one of 5 pN [64].

The energy required to generate the torque in F_1 is about 8×10^{-20} J for a 120° rotation step. The free energy that is provided by the hydrolysis of one ATP that is hydrolyzed for a 120° rotation is about 9×10^{-20} J. In other words, the ATP synthase, the rotary motor that Nature has designed to synthesize ATP, indeed runs at about 90% efficiency. The efficiency may even be higher if viscoelastic mechanics of the ATPase-actin-construct are considered [65,66]. Experiments and theoretical analysis suggest that the torque that is generated by the synthase exceeds 40 pN nm and was estimated to be more in the range of 50 ± 6 pN nm. The relatively small variations in the torque output of the motor at different loads imply a soft, elastic power transmission between the two parts of the motor, F_0 and F_1 which seems to be an essential feature to allow this bio-motor to perform under such high turnover rates.

3.3. Nanoelectromechanical systems

Soon after the rotary mechanism of the ATPase was demonstrated by Yoshida and others, researchers started to investigate its application in the nanomechanical world. Carlo Montemagno and his co-workers pioneered in this work and reported the "powering [of] an inorganic nanodevice with a biomolecular motor" in *Science* in 2000 [67] (also see [68]). The group used a similar minimal motor structure of the F_1-ATPase from the thermophilic bacterium, PS3, as was used in the original approach by Noji et al., with a subunit composition of $\alpha_3\beta_3\gamma$. Using electron beam lithography, an array pattern was etched on 25 mm coverslips which were then patterned with metal substrates like gold, copper or nickel. Synthetic peptides containing polyhistidine-tags as described above were allowed to attach to the metal-coated coverslips.

The strength of the attachment of the polyhistidine tags to the different metal substrates were tested using laser tweezers that 'pulled' on microspheres that were covalently attached to the his-tagged peptides. Nickel was found to be the most promising metal substrate candidate and therefore, in the following paper [69], the group presented a method to generate nanofabricated Ni-posts of a diameter of 50–120 nm and a height of 200 nm. Ni-posts were chosen as a 'pedestal' onto which the ATPase-motor was positioned. This was deemed necessary to prevent problems associated with 'dragging' the 'propeller' unit in close proximity to the base of the motor, especially in view of the small dimensions of the ATPase motor of only about 15 nm 'height'. The nickel propeller rods that were generated were optimized for optical detection and minimal friction during the rotation process. The propellers were then coated with biotinylated, His-tagged peptides that allowed attachment of the peptides to the Ni-rods, while the biotinylation brought about the interaction of the propellers with streptavidin-modified F_1.

Functional NEMS were constructed by sequential addition of the individual components: F_1-ATPase was biotinylated at its γ-subunit through specific modification of engineered cysteine residues in the appropriate position. The biotinylated F_1 was placed onto the Ni-posts by virtue of the histidine-tags that were located at the β-subunits of the constructs. Streptavidin was bound to the biotin molecules attached to the γ-subunit and finally the Ni-propellers decorated with biotinylated peptides were added and assembled onto the motor. A cartoon depicting the design of these first NEMS is shown in Fig. 7.

Finally, and most ingeniously, the F_1-part of the NEMS was further modified to contain a chemical switch that allows the system to be turned on or off, without having to run the motor out of its power source, ATP [70]. Using computational design methods [71–73] a binding site for Zn^{2+} ions was designed, that, when complexed with Ni^{2+}-ions inhibited rotation. Upon addition of other chelating agents, Ni^{2+} could be removed and the motor

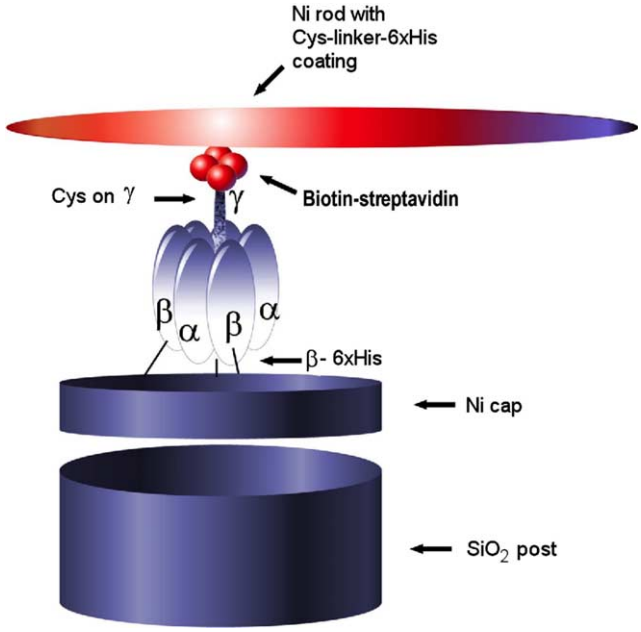

Fig. 7. A cartoon of a first nanoelectromechanical device [67]: a minimal F_1 complex containing $\alpha_3\beta_3\gamma$ was immobilized via His-Tags onto Ni-post structures of a diamter of 50–120 nm and a height of 200 nm. Subunit γ was biotinylated and using the biotin–strepavidin–biotin construct, a spacer was formed that bound to prefabricated Ni-rods that have been covered with His-tagged oligopeptides. Upon addition of ATP, the ATP-driven rotation of γ can be visualized as a rotation of the Ni-rod.

starts up again. A little prior to that, Hisabori and co-workers reported a very similar approach to turn the motor on and off which is based on redox reactions [74]. They introduced a chemical switch in form of two cysteine amino acid side chains in close vicinity into the γ-subunit. In the presence of chemical oxidants like $CuCl_2$, disulfide bonds are formed between the two sulfhydryls that inhibit rotation of γ. Addition of reducing agents like dithiothreitol, or thioredoxin, an enzyme that is a natural regulator of the ATPase from chloroplasts then reinstated the rotary motion of the ATPase.

4. Outlook

At the time Montemagno and his group performed their initial, groundbreaking experiments that showed that nanoelectromechanical devices using biomolecules like the ATPase were feasible, their group was located at the Cornell University in Ithaca, NY. Some 40 years prior to that, from this very same university, the researchers Efraim Racker, Harvey Penefsky and their co-workers reported that they had isolated a soluble 'factor' from beef heart mitochondria that hydrolyzed ATP [75]. They reported that this 'factor' could restore ATP synthesis in mitochondrial membranes that had lost this ability during isolation. They called this interesting protein the 'Factor 1' or F_1 for ATP synthesis. The research on the F_1-ATPase and the F_0F_1-ATPsynthase was born. It took more than 40 years of worldwide, high-powered, basic research to get to the point where we now are, which is understanding significant parts of the molecular mechanism that are the basis of Nature's designs.

Now, first steps for the ATP synthase into the 'nano-world' have been successfully performed. Soon, it is likely that similar results will be obtained for the linear motors as well. At some point they may be used on nanoworkbenches to transport substrate from one 'nanoside' to the other, as pumps or mixers in nanoscale chemical reaction chambers, as switches to modulate flow of fluids or electricity or even as motors to drive nanosubmarine structures. 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.

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