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KINESIN: MECHANICS OF THE MOLECULAR MOTOR

D.A. Shestakov

Institute of Mechanics, Lomonosov Moscow State University, 1 Michurinsky prospect, Moscow, 117899, Russia, e-mail: unasalus@yandex.ru

Abstract. Molecular nanomotors provide a variety of mechanisms of biological motility. Kinesin is one of the most common and promising for study proteins. It serves for the intracellular transport of cargos along the specific molecular pathways - microtubules formed by protein tubulin. Kinesin interacting with tubulin is involved in cell division and the movement of flagella and cilia. Kinesin is in many ways similar to another motor protein – myosin. Their motor domains have a similar structure. Mechanochemical cycles of both proteins are based on ATP hydrolysis as a source of energy for the mechanical transitions. Myosin interacts with the actin filaments and plays a key role in muscle contraction. On the other hand, kinesin has some significant differences from myosin in general structure and biomechanical interactions. Nowadays, we know how kinesin binds to tubulin. We know how kinesin moves processively along the microtubules covering distances as long as thousands of its elementary working steps. We also can say something about its power and speed. At the same time, many mechanical aspects of the kinesin motor are still under discussion. Such aspects include some details of the mechanism of information transfer from one globular head to the other, the existence of intermediate states in the biomechanical cycle, the nature of the reverse steps of kinesin. To clarify these and other details further, biomechanical researches are carryied out in current works. Their authors create amazing mathematical models using the methods of continuum mechanics, theoretical mechanics and mathematical statistics. This review aims to gather all up to date information on kinesin and its interaction with tubulin in the context of modern biomechanics.

Key words: kinesin, tubulin, molecular motor, nanomechanics.

STRUCTURE AND A BRIEF HISTORY OF STUDIES OF TUBULIN AND KINESIN

Kinesin is one of the most interesting and attractive motor proteins. Knowledge of its structure and mechanics is required for the understanding of various types of molecular motility, primarily the mechanism of the fast transport of organelles along the intracellular highways - microtubules composed of the tubulin protein.

Microtubules were discovered a long time ago [113]. However, they did not have names at that time. They looked like a strange divergent beams of illuminated road network of the night city as one can see it from the window of an aircraft. This was due to a very low image resolution available at the moment. Tubulin was named many years later and identified as a building material for the microtubules [9, 35]. Finally, structure of the blocks of these roads are paved - tubulin heterodimers were obtained with the atomic resolution in 1998 [80]. Heterodimer is a very stable non-covalently associated structure consisting of α and β-tubulins - monomers with the extreme structural similarity (approximately 450 amino acid residues, 55 kDa). Each of these monomers contains a molecule of guanosine triphosphate

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Dmitry A. Shestakov, Ph.D., Senior Researcher, Laboratory of Biomechanics, Moscow

(GTP) [70]. Hydrolysis of the GTP contained in the β-tubulin takes place during the assembly of the dimers into long thin filaments - protofilaments. Therefore, β-tubulin contains GDP (guanosine diphosphate) in the microfilaments [25]. Small differences in the structure of α- and β-tubulin affect their interaction with kinesin. It is known that most types of kinesin walks on β-tubulin monomers [74] from the cell center towards the periphery while a few representatives of the kinesin family walking in the opposite direction are more promiscuous and also form complexes with α-tubulin [110]. The end of the microtubule which encloses β-tubulin is called the plus end. The other end is capped with α-tubulin and this one is called the minus end. Microtubules are mainly stabilized at the plus end while their growing and disassembly occurs presumably at the minus end [12].

Microtubules are cylinders formed by connected side-to-side protofilaments. Since the connection is made with an axial shift, tubulin of microtubules forms triple helix with the offset of 0.92 nm [2] (Fig. 1). Although microtubules *in vitro* consist of 10-16 protofilaments [17, 75] and in some cases they can form clusters [109], yet standard microtubule is composed of 13 tubulin protofilaments [102]. The assembly of microtubules begins in the centrosome from which they grow in the direction of cell periphery. Their plus-ends are pointed towards cell membrane [1]. Actin filaments and microtubules serve for a more slow and accurate delivery of "goods" within the cell. Microtubules along with actin and the intermediate filament proteins form so called cell cytoskeleton.

In addition to their transport function, microtubules also play an important role in cell division. They form the mitotic spindles which are attached to the kinetochore - the special structure that is associated with the chromosome. During mitosis, the two spindles move apart to form new centers of the newborn cells [42]. Kinesin is involved directly in this process as well.

Thus, kinesin provides the most different kinds of biological motility along with myosin and other motor proteins. First micrographs of the cytoskeleton with the clearly visible cross-bridges connecting microtubules with the cell organelles were obtained in the early 1980-ies [45]. It has been suggested at the moment that some of these bridges are formed by intracellular transport proteins [45, 46]. In 1985, the first of them was identified by Brady [10] and Vale [106] as a protein that serves for the fast axonal transport. It was called kinesin. A year later, Kuznetsov and Gelfand showed that this protein is an ATPase with the rate of ATP hydrolysis that increases dramatically in the presence of tubulin as well as the presence of actin accelerates myosin ATPase [65].

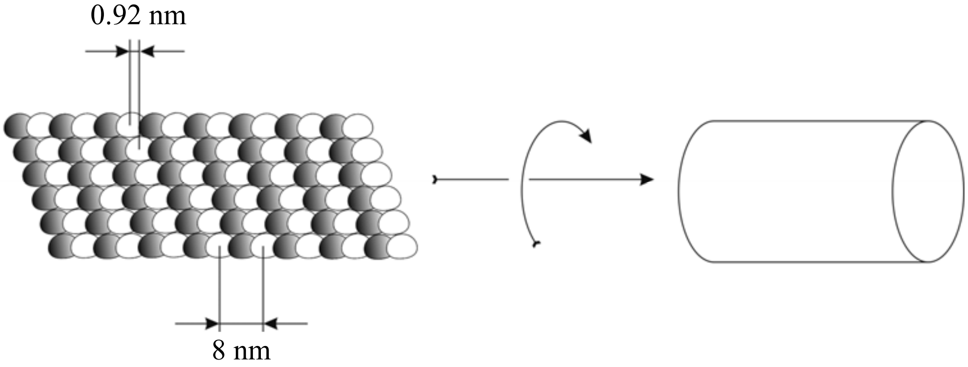

Fig. 1. The scheme of the microtubules assembly. Protofilaments formed by the dimmers of α-tubulin (white monomers) and β-tubulin (shaded monomers) are shown at the left side of the picture. The direction of enrolling the sheet of protofilaments into a microtubule (assembly) is shown by the curved arrow at the center of the picture. The size of one dimmer is shown at the bottom left. The size of the step of axial repeat induced by the shift of the protofilaments is shown at the top left

The structures of the motor domains of human and rat kinesin were determined by X-ray crystallography studies a decade later [62, 88]. This type of kinesin was called conventional kinesin or kinesin-1 because it is the most easily extractable and thus available for the experiments [14]. It later became clear that kinesin hydrolyzes one molecule of ATP per one working step [21, 90]. The step size is equal to 8 nm which is the distance between the tubulin dimers [98]. Now, we know that kinesin molecule is capable of processive moving along microtubule with a speed of 900 nm/s [91] for the distances measured with micrometers [104], i.e. hundreds of kinesin working steps.

As new subtypes of proteins interacting with microtubules were discovered, the need for systematization and classification of these proteins arose. Thus, all kinesin-like proteins were organized into the kinesin superfamily consisting of 14 classes from kinesin-1 to kinesin-14. This new classification was approved and adopted in 2004 [22, 68].

All types of kinesin have globular heads - motor domains (≈ 40 kDa, 350 amino acid residues) which contain the tubulin binding and active (where ATP or products of its hydrolysis bind) sites. The distal parts of all kinesins are rigid coiled-coil parts of the heavy chains and the light chains which usually form a tail - cargo-binding site [49]. Very flexible hinge connecting the head with the rest of the heavy chains is called the neck linker. Most kinesins are move cargo from the cell center towards the periphery. They are called N-kinesins because of the position of their motor domain interacting with tubulin. In N-kinesins, it is placed at the N-terminus of the protein primary structure. One can guess that C-kinesins got their name because its motor domains located near the C-terminus of the molecule. These kinesins are members of the kinesin-14 class (also known as Ncd-protein) and they are carrying cargos in the opposite direction - toward the center of the cell [87, 92]. Finally, those kinesins that have their motor domains in the middle of its primary structure are called the M-kinesins (kinesin-13 family). These motors are involved in the disassembly of the microtubules [77]. Of particular interest is kinesin-5 that forms heterodimers with four motor domains located on both sides of the dimer. It is capable to attach to two different microtubules simultaneously and push them against each other. This is required to pull apart two nuclei of newborn cells when the process of cell division occurs [30, 57]. Structures of kinesin tails may vary widely depending on the kind of goods they intended to carry on [95]. Different types of kinesins are involved in two different types of intracellular transport: fast transport of organelles along the axon and slower transport of other organelles and cytoskeletal proteins. This leads to the other distinctions in structure [47]. There are some evidences that kinesin-1 participates in both fast and slow types of transport. The mechanisms of its speed control are under discussion [101].

Kinesin is also involved in the movement of cilia [81] and flagella [6]; it carries the fluorescent proteins within the melanophores [84]. It can be found in plants and fungi. Today, we have about 70 atomic structures of various types of motor domains of kinesin from almost all of his family branches.

SIMILARITIES AND DIFFERENCES BETWEEN KINESIN AND MYOSIN. BIOCHEMICAL AND MECHANICAL CYCLES

Myosin is another important motor protein. Just like kinesin that interacts with tubulin, myosin interacts with actin playing a key role in muscle contraction and other types of biological motility [93]. Both motors, myosin and kinesin, are extremely efficient and ensure energy conversion efficiency of 50 % and higher [7, 97]. Muscle myosin-2 and non-muscle myosin-5 and some other members of myosin family also have two globular heads just like the main subject of this review, conventional kinesin-1. As kinesin-1 walks stepping with its heads on tubulin monomers, myosin-5 moves along an actin filaments [94]. Both proteins transport cargoes within the cell. Both proteins hydrolyze ATP. One can imagine an intracellular “parcel” which is delivered by the “high-speed train” (kinesin) along the tubulin “railways” to the intermediate station. At this station, the good old humble "lorry" (myosin-5) is waiting to transport the “parcel” to its final destination along the “dusty country road” (actin) [11]. At the same time, myosin-5 has twice larger motor domains than that of kinesin and its steps are four times longer, but its velocity is nine times lower [16]. However, a question arises: are myosin and kinesin indeed so very similar and, if yes, to what extent?

At the first glance, one can see the structural similarity of the motor domains of both proteins, in particular their active centers in which the cycle of ATP hydrolysis occurs including ATP binding and release of the hydrolysis products, ADP and inorganic phosphate [62]. Energy of the ATP hydrolysis is used for the mechanical changes in the motor proteins. So it is quite understandable that the biochemical transitions in myosin and kinesin are directly related to their conformational changes. Their globular heads must contain some special switches that are sensitive to the presence of nucleotides and its magnesium ions. These switches differentiate α, β and γ-phosphates and are capable to transmit “information” about their presence to the other parts of motor domains causing conformational changes. There are four conservative motifs in the structure of these sensitive regions. They form the flexible phosphate P-loop involved in the process of phosphate binding and switches mentioned before (switch I and switch II) [87, 107]. These motifs of kinesin and myosin molecules consist of the same amino acid residues and also have very similar fold. They are attached to the similar seven-stranded β-sheets serving as the frame for the whole motors. All of these explain the correlations in the force-generating mechanisms of myosin and kinesin [61]. The authors of [63] believe that the similarity in structure of both proteins indicates that they have common ancestors - G-proteins which participate in the transmission of the external stimuli into the cell and use the energy of GTP hydrolysis. Summarizing, one can conclude that small changes in the nucleotide-binding pockets of the motor proteins can be translated into large-scale mechanical transformations. In kinesin, this translation results in packing of the neck-linker hinge between the globular head of the molecule and its helical “neck” [83]. In myosin, the translation results in tilt movement of the converter domain that causes even more prominent tilt and the so-called lever arm [51, 82].

Despite all the similarity of myosin and kinesin, there are striking differences in dynamics of their interaction with actin and tubulin respectively. Biochemical cycle is the same: ATP binding to the empty nucleotide pocket, then hydrolysis, then phosphate release and finally the release of ADP. However, conformational changes in myosin related to force generation are accompanied by the phosphate release [5, 71]. On the contrary, kinesin force generating step takes place along with the binding of ATP [28, 40, 44]. Opening of myosin nucleotide pocket strengthens actin binding [50]. When myosin binds ATP myosin and detaches from the actin filament and enables closing of its nucleotide pocket and hydrolyze of ATP, molecule mainly is in detached state. The release of hydrolysis products is hampered when the pocket is closed and myosin binding to actin is very weak and reversible. Strong binding to actin is only possible after the opening of the nucleotide pocket which involves the structural changes in the actin binding site of the myosin head. Strong binding to actin allows the pocket to open widely and release inorganic phosphate and then ADP. The phosphate release is accompanied by force generation (as mentioned above) and opens the way for ADP release [34]. Kinesin behaves quite differently [1] (Fig. 2).

The release of inorganic phosphate leads to a weakening of its binding to tubulin [23]. This provokes kinesin detachment from tubulin with the subsequent ADP release. The last step opens the tubulin binding site and promotes the strong binding of a free kinesin head to

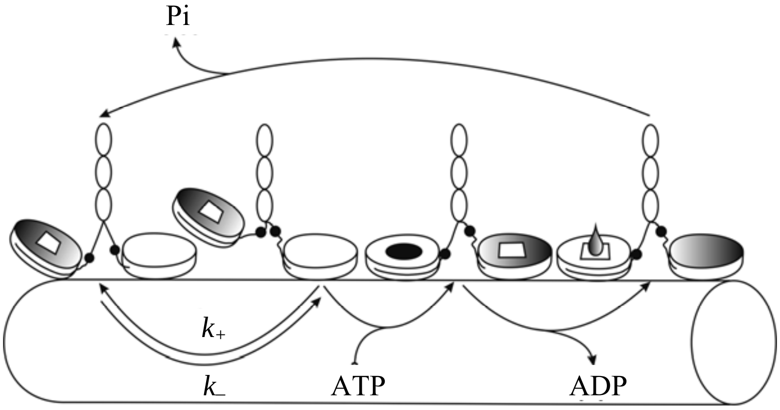

Fig. 2. Biomechanical cycle of kinesin walking along a tubulin microtubule is illustrated. The direction of kinesin walking is from the left to the right. Transitions between the different states of the kinesin molecule are shown by arrows. ATP binding and release of the products of its hydrolysis are also shown by arrows. The first and the second states of the cycle are in the dynamical equilibrium that is shown by the arrows of opposite directions and by the corresponding rate constants. One of the heads of kinesin dimer (mentioned in the text as the front or the leading head) is shown white in the all states of the cycle. The other head (mentioned in the text as the rear or the driven head) is shown as gradually shaded. ATP molecule is shown on the motor domains as a black ellipse, ADP molecule is shown as a white rectangle and inorganic phosphate (Pi) is shown as a gradually shaded drop. The positions of the zipped or unzipped neck linkers are shown by the black curves with the small black circles

the microtubule [59]. After that, the ATP binding is followed by ATP hydrolysis and then force generation through the neck-linker mechanism [86]. This opens the nucleotide pocket and enables phosphate release. The strongest binding of myosin to actin is achieved in a state that corresponds to *rigor mortis*, i.e. in the absence of nucleotides in the pocket. At the same time, kinesin strongest association with tubulin occurs in the presence of ATP [22]. Interestingly, Taylor discovered the main phases of the myosin-actin ATPase (with Lymn) and twenty years later became one of the principal investigators of the kinetics of the kinesin-tubulin interactions.

THE MECHANICS OF KINESIN WALKING ALONG TUBULIN

Let me apologize to the reader for the use of a rather strange terminology that the author has not invented. In some cases, this terminology may lead to misinterpretations. Traditionally, those parts of the kinesin molecule that bind tubulin, are called “heads” whereas the part which carries the load is called “tail”. At the same time, the obvious analogy for the movement of kinesin along the tubulin microtubules is walking. Thus, one may have a quite understandable temptation to refer to the motor domains as “legs” and to the light chains holding the cargo as “head”. As a result, there is some confusion that leads our mind’s eye to a picture of an absurd creature walking on the two heads with its tail up. Still, this is the image that really helps to decipher this section of the review. And as if that was not enough, many authors use the term “hand-over-hand” to describe the manner of the kinesin walking. After all, one cannot be dramatically surprised by the fact that the motor domains appear as heads, feet and hands within the same article [55]. To avoid the final confusion in the description, all of these variations will remain outside the brackets. Kinesin motor domains will be referred as heads till the end of this review.

So, how exactly a two-headed kinesin molecule walks along the microtubule? As mentioned above, each force generating cycle of kinesin is driven by hydrolysis of one ATP molecule. One step propels the molecule at a distance of 8 nm. For several years, various authors argued for the existence of some intermediate “half-steps” [20, 79]. However the evidence that was found in support of these hypotheses has been later proved to be a result of some inaccuracy in the interpretation of the experimental data [13, 111]. Therefore, today we may not to pay any serious attention to this idea.

A question about the type of kinesin “bearing” or allure was a great deal of controversy. There were three options that seemed to be quite possible. 1) Symmetric walking: one head crosses over another, the leading and the driven heads are swapped at each step, the steps are symmetrical and equivalent. 2) Asymmetric walking: mostly the same as in the first case, but the steps of the different heads are mechanically different. 3) Limping: one head is the leading head all the time and the other head is pulling to the leading one at the each step.

The experimental data was seemed to be contradictory at the first glance and provided arguments in favor of any of the listed options. It was found that kinesin-1 does not turn its rigid coil while walking [52]. This was the evidence in favor of the third option. On the other hand, the experiments with the fluorescent markers showed that the step size for an individual head is 16 nm. Hence, each head has one step for one tubulin dimer [115]. This was a serious argument in favor of the first option. Finally, the second option could explain both phenomena. Some data also showed that kinesin allegedly limp during the walking [3, 58].

The picture began to clear with new data that are more accurate. Block proposed the so called “Consensus” model in 2007. He designed this model to reconcile the different positions [8]. Seven years later one can say that disputes concerning the matter are now reduced to the refinement of some subtle details.

First let us consider the two states of a kinesin molecule in which the front (leading) head is free of nucleotides and strongly bound to tubulin, while the rear (driven) head binds ADP and is either disconnected from tubulin or weakly attached to it. Recent experiments have shown that these two states are in the dynamic equilibrium with the equilibrium constant of about $k_+/k_- = 1.4$ (here k_+ is a rate constant of the attachment of the ADP-bound head to tubulin, see Fig. 2) [103]. The attachment of the second head produces mechanical tension in the neck linker (see section 2). When it is detached from the microtubule, the neck linkers of the two heads are close to each other [103]. At the beginning of the new millennium, a hypothesis was proposed about the strain of the linker on the rear head that prevents the front head from the ATP binding [41, 85]. The recent experiments with fluorescent labels suggest that the probability of the ATP binding by a head strongly attached to tubulin increases when the two neck linkers of the two heads are geometrically close to each other. This position is impossible until the rear head is bound to the microtubule [103]. Therefore, its detachment makes the next step of the cycle available. Here, one can see that the “information” about the current state of one motor domain of the kinesin dimer may transfer to another head mechanically.

When the rear head detaches from tubulin at the next phase of the cycle, the front one binds ATP. This results in the energy release that is required for the almost irreversible repacking of the neck linker of the leading head. The neck linker zips on the surface of the head tightly [4, 83]. This zipping provides the shift of the detached head by 1-2 nm toward the plus end of microtubule. That shift is considered to be the force-generating step of kinesin. Now, the detached head is in a good position for the finding a suitable attachment site. There is a nonzero probability of the reverse attachment to the previous site. But, this probability is negligible as zipped neck-linker of the other head produces a mechanical tension that makes the front tubulin monomer energetically more favorable for the attachment [99].

Although the free head undergoes the Brownian fluctuations, it turns out that there is an energetic barrier, a kind of ratchet which prohibits its back movement. This phenomenon has been described in the scientific literature as a thought experiment called “Brownian ratchet” [31]. It is a “Maxwell’s demon” of some kind (Brownian ratchet as a term to describe the actin-myosin interaction was proposed by Huxley [54]). Note that the whole process starting with the ATP binding to the front head and ending with the reattachment of the former rear head to the new tubulin monomer occurs much faster than the other steps of the kinesin mechanochemical cycle [13]. So, the probability for the both heads to be simultaneously detached from tubulin is negligible. This fact provides plausible explanation for the high processivity of the kinesin motor.

At the next step of the walking cycle, both heads are attached to tubulin. Although the former rear head still contains ADP, the former front head is now associated with the ATP molecule. This produces a mechanical tension that prevents the ATP binding to the head that contains ADP even after its release. The tension persists until the ATP bound to the other head is not hydrolyzed [41]. Phosphate release by the former front head completes the walking cycle.

Both heads take equal part in the proposed scheme of walking and change their roles consecutively. This scheme shows why kinesin is capable of processing movement along the microtubules for such a long distances and also why it keeps moving in the same direction along the same protofilament.

KINESIN UNDER LOAD: FORCE AND VELOCITY

Different approaches were implemented to study the mechanical properties of kinesin. Optical trap experiments were used to analyze the behavior of single molecules. Mechanical load was applied to tubulin filaments to study the behavior of kinesin ensembles and the ability of kinesin to step backward. Let us consider these experiments in detail.

First, researchers had a natural desire to determine force of a single molecule of kinesin-1. This was achieved for example by attaching some sort of dynamometer to the microtubule via the latch with known stiffness. Then, kinesin molecules glued to the substrate were allowed to pull the microtubule [76]. Polystyrene micron beads were used in the other experiments. Kinesin molecules were attached to the surface of the bead. The bead was placed in an optical trap that is capable to hold a particle in place by the light pressure of the laser beams. The microtubule was placed in the vicinity of the bead. Thus, single molecules of kinesin were attached to the microtubule and generated force. This force induced the deviation of the position of the bead that was measured by the optical trap [108]. Recently, two-dimensional optical traps with the feedback were developed [67]. When a kinesin molecule moved along a microtubule, the optical trap generated force acting in the opposite direction and reducing the speed of kinesin walking until it stops. Different research teams used different methods to obtain the same result - the force that is needed to stop a single kinesin molecule or in other words the maximal force of single kinesin molecule is equal to 5-7 pN [20, 60, 108].

Dependence of force on the velocity of kinesin walking can be described in the general form using a three-parameter equation:

$$v = v_0\left(1-\left[\frac{F}{F_s}\right]^w\right), \tag{1}$$

where v_0 is the maximal velocity under zero force (≅ 650-800 nm/s) and F_s is the maximal force that stops kinesin molecule (stall force) [64]. Finally, w is the curvature parameter that can vary for the different series of experiments with different kinesin samples. As an example, the force-velocity dependence for the kinesin-1 extracted from the bovine brain is almost linear ($w \approx 1$, [53]). At the same time, the data obtained for the squid kinesin-1 is well approximated by the curve with the parameter $w = 2.5$ [108].

Interesting reverse experimental technique was invented recently to measure the force and velosity of kinesin. Instead of attaching kinesin molecules to a bead, a magnetic bead was attached to the plus end of a microtubule. Kinesin molecules glued to the glass started to interact with the microtubule pushing it away from its plus-end. The magnetic field was applied to the bead producing the force pulling to the opposite direction. In this way, the force and velocity of kinesin were measured. To estimate the average distance between the kinesin molecules participating in the interaction with tubulin, the direction of the magnetic field was reversed. Thus, the microtubule bent at an angle of almost 90 degrees in the exact place where it was attached to the first molecule of kinesin. The rest of the microtubule continued to move until it drove past the next active kinesin molecule moving the point of the bending. Thus, the force–velocity dependence per one molecule was evaluated. The results of these experiments can be described by the curve (1) with the parameter $w = 1.8 \pm 0.4$ that supports the idea of the independent work of individual motors [29].

Many experiments were focused on the phenomenon of kinesin back steps. It is well known for long time that kinesin can occasionally make a reverse step [97]. However, experiments with external load have shown that the higher the load the greater the probability of the reverse step [13, 79]. When the load exceeds the stall force (above 10 pN), kinesin is appeared to show a slow processivity, i.e. step-by-step backward walking for relatively long distances [13]. This fact leaded the authors to the assumption that the reverse movement of kinesin is accompanied by a complete reverse of its biochemical cycle that is associated with ATP synthesis in the motor domain. It is known that at certain conditions ATP synthesis takes place for example in other molecular motor F_1 [56].

Experiments were performed that allowed the estimation of the effect of ATP concentration on speed, strength and processivity of the back steps [13, 18, 32]. Many authors have questioned the hypothesis of reversed ATPase [8, 39]. All this resulted in a long-time discussion that provided more arguments against the hypothesis. It was found that even in the absence of any nucleotide kinesin performs consecutive back steps if the sufficient external force is applied. Moreover, large external force may induce processive forward walking in the absence of nucleotides [114]. These and other data have provoked researchers to propose different new models to explain the inverse steps of kinesin [15, 18, 55]. The most straightforward idea is that a large external force compensates the mechanical tension generated by the neck-linker zipping to the ATP-bound head (see section 3). Thus, the result of Brownian search becomes shifted to the reverse transition. Then, the probability of ADP release becomes higher. This release causes the rear head to bind tubulin strongly and leads to the next phase of the reverse step cycle.

It should be recognized that the discussion about the nature of the back stepping mechanics is not yet over.

MATHEMATICAL MODELS

Now let me consider briefly a few mathematical models used to describe the mechanics of the kinesin motors. In the *in vitro* experiments mentioned above, it is easy to observe directly the velocity of moving microtubules or microscopic beads covered by kinesin molecules. However, it is much more difficult to estimate the force developed by the kinesin molecules (see (1)). This sometimes leads to the solving of non-trivial mechanical problems.

In the experiments [36], the minus end of the microtubule was attached to the glass plate. Kinesin molecule interacted with the microtubule near its plus end developing a compressive force. Since this force exceeds the Euler's critical buckling load, microtubule losed its stability and bent (Fig. 3). To calculate the force that causes the observed bending of the microtubule Euler's bar formula was used and the derivations of the bending equation for the loaded axially compressed rod were solved. The equations can be found in [66]:

$$\theta''(s) = \beta^2 \sin\left(\theta(s) - \varphi_f\right), \text{ where } \beta^4 = |F|^2 / (EI)^2. \tag{2}$$

Here, s is the arc length along the tubulin filament, θ is the tangent angle of the tubulin filament (see Fig. 3) and φ_f is the angle of the force F with respect to the parallel axis. EI is the flexural rigidity of the microtubule determined experimentally by its changes in shape due to the thermal fluctuations [37].

It is known that the equation (2) have exact solution that can be written by the incomplete elliptic integrals of the first kind [66]. After some intermediate calculations with the use of the boundary conditions, this solution allows one to evaluate the Euler's critical buckling force and thus to estimate the value of the real force F developed by a kinesin molecule [37].

Another way to determine force of a kinesin *in vitro* is to increase the viscosity of the solution within the experimental cell gradually until the velocity of walking drops to almost immeasurable value. To evaluate the force, one has to solve the problem of motion of the cylinder (microtubule) in a viscous medium (see [96], for example). However, solving the standard equations is not enough because the substance that changed the solution viscosity brings some non-Newtonian properties to the fluid. Fortunately, the fluid with the shear-dependent viscosity can be described as "generalized Newtonian fluid":

$$\overline{\sigma} = \eta \overline{s}, \qquad \overline{s} = \nabla u, \qquad \eta = \eta\left(|\overline{s}|\right). \tag{3}$$

These equations are written for the plane perpendicular to the cylinder axis. The shear tension vector $\overline{\sigma}$ depends on the velocity gradient ∇u via generalized viscosity η that is a function of the shear rate. This complex system of equations cannot be solved analytically. However, a numerical solution is well approximated by the formula for the dependence of the force on the velocity [53]:

$$F(v) = c_0 v \left(1 + \left[\frac{v_0}{v}\right]^{0.75}\right). \tag{4}$$

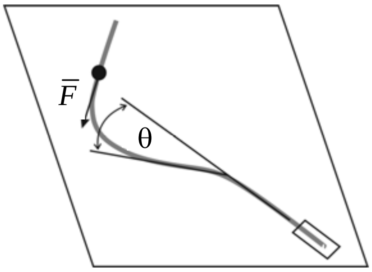

Fig. 3. Measurement of the force developed by a single kinesin molecule is illustrated. The thick grey curve represents the tubulin microtubule which is attached to the glass at the minus-end. Kinesin dimer (black circle) is associated with the microtubule and develops the compressing force F (black arrow). The angle θ between the initial direction of the microtubule and the current tangent is also shown (see Eq. (2))

Two types of mathematical models are mainly used to describe the biomechanical cycle of kinesin. Firstly, there are statistical models of Markov type. In these models, the set of all biochemically and (or) mechanically different states of kinesin-tubulin complex (usually more complex and detailed than that shown in Fig. 2) is represented by the probability distributions. Rate constants are used as probabilities for the transitions from one state to another. Typical system of equations is as follows:

$$\frac{d\rho_i(t)}{dt} = \sum_i \left[r_{ji}\rho_j - r_{ij}\rho_i \right], \tag{5}$$

One can easily find systems of that kind in the model papers devoted to the actin–myosin interaction cycle. In the system (5), ρ_i is the probability for the kinesin molecule to be in a state i while r_{ij} is the rate constant for the transition of kinesin from the state i to the state j. One can additionally assign a linear coordinate along the microtubule and make the rate constants dependent on this coordinate. One can also introduce the dependence on the concentrations of ATP, ADP and inorganic phosphate into a model. Arrhenius equation for the direct and reverse transition rates and conservation laws complete the system (5). Among other possibilities one can then search for the stationary solution of the system of equations (as the authors of [71]) and calculate the macroscopic characteristics of the model deriving dependence of force on any variables such as the concentrations of ATP and products of its hydrolysis.

Anyone who constructs a model of Markov type faces one major difficulty that lies in a large number of parameters (in particular the rate constants) which can only be estimated approximately but cannot be measured directly.

In the second type of models, their authors describe different states of kinesin-tubulin cycle as a set of energetically equilibrium positions separated by the energy barriers. In the models of that type, the mechanical tension between the two heads of kinesin is transmitted by an elastic spring [27]. This leads to the Langevin equations:

$$\Gamma \dot{x}_n = -\partial_x V(x_n) - F + K\left[x_m - x_n - l(t) \right] + \xi_n(t), \tag{6}$$

where m, n = 1, 2 are the numbers of kinesin heads (if $n = 1$ then $m = 2$ and *vice versa*). Also x is the axial coordinate, Γ is the frictional drag coefficient, V is the periodic potential, K is the spring stiffness, l is the equilibrium position of the head (0 or 8 nm before and after a step, respectively), ξ is the Gaussian white noise, i.e. the value of the random Brownian force. The system (6) was solved by using the Monte Carlo method [27].

CONCLUSIONS

Although some major breakthroughs were made recently in the studies of the kinesin-tubulin interactions, there remains a large scope for the future researches. The mechanics of kinesin back steps is not clarified. We are still waiting for the new kinesin–tubulin complexes in different biochemical states to be crystallized. We still do not know how the collective interaction between the kinesin molecules of the different or the same types occur. How the continuous movement of cargoes along the microtubules in alternating directions is organized? How exactly a kinesin molecule keeps a constant direction of motion along the one tubulin protofilament? How one can combine the Brownian ratchet model with the mechanics of force generating step? Does the zipping of the neck-linker provide enough energy to perform this step? These are few of many questions waiting for the final answers.

Further studies of kinesin are extremely important. As one of the major neuronal proteins it plays a significant role in the mechanism of Alzheimer disease [38, 43]. Targeting kinesin–tubulin interactions during the process of cell division is one of the ways to develop new methods of cancer treatment [69, 89]. Kinesin-tubulin walking mechanism now serves as one of the prototypes for the newly constructed artificial and half-natural molecular motors which are intended to use for the intracellular drug delivery [26].

Let us all to wish a good luck to anyone who chooses kinesin as the area of expertise and future research. To learn more about the different aspects of kinesin mechanics the author recommends more comprehensive reviews [8, 23, 48, 73, 100, 105, 112].

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