УДК 665.939.14

K.J. BÖHM, R. STRACKE, E. UNGER

Institute of Molecular Biotechnology,
Beutenbergstrasse 11, D - 07745 Jena, Germany
E-mail: kboehm@imb-jena.de; Web: http://www.imb-jena.de/~kboehm/

MOTOR PROTEINS AND KINESIN-BASED NANOACTUATORIC DEVICES

Eukaryotic organisms synthesize diverse motor proteins converting chemical into mechanical energy. Among them, both rotary (e.g., ATP synthase) and linear motors are found. Linear motors comprise highly specialized proteins moving along nucleic acid filaments (in the case of e.g., RNA polymerase) or cytoskeletal filaments. The present paper provides a brief overview on cytoskeleton-associated motors (myosins, dyneins, and kinesins) and summarizes results contributing to elaborate a basic configuration for constructing a kinesin-driven motor device, suitable for e.g. a controlled displacement of objects or specific substances over millimetre distances with nanometre precision.

Introduction

Nature has evolved highly specialized motor proteins (*synonym*: mechanochemical proteins), which move along stationary filamentous tracks or rotate around a defined axis. During movement the motor proteins convert chemical into mechanical energy, required to realize diverse motility processes, such as cell locomotion, intracellular organelle transport, muscle contraction, genomic transcription, and bio macromolecular synthesis.

In analogy to technical systems, the motor proteins can be classified into two groups: *rotary motors*, including flagellar motors [1, 2] and the ATP synthase [3–5]; *linear* (forwards propulsive) *motors*, including RNA polymerases [6–11] and cytoskeleton-associated motors.

The cytoskeletal motor proteins comprise a great number of different types of myosins, dyneins, and kinesins. Unlike the RNA polymerase which walks along DNA strands, these motors move on the surface of proteinaceous filaments of the so-called cytoskeleton. Native myosin, dynein, and kinesin molecules reveal distinct globular heads containing both a catalytic centre, and the filament-binding site at the one end and a cargo binding site at the other one. Hydrolyzing energy-rich nucleoside triphosphates (preferentially ATP), the catalytic centre delivers the energy for movement. In dependence on their main functional activities, cytoskeletal motors can be divided into:

1) motors involved in intracellular transport processes (kinesins, cytoplasmic dyneins, cytoplasmic myosins, *e.g.*, myosin V, myosin VI);

2) motors responsible for cell locomotion and cell surface transport (axonemal dynein);

3) motors for muscle contraction (myosin II).

Another classification made on the basis of the type of cytoskeletal filament which these motors move along results into two subgroups:

1) microtubule-associated motors (kinesins, cytoplasmic and flagellar dyneins);

2) actin filament-associated motors (cytoplasmic and muscle myosins).

One of the best characterized cytoskeletal motor proteins is the kinesin. Moving unidirectionally over relatively great distances without being released from the microtubule surface, it seems to be well suited to be involved in the development of a cell-free linear motor device. Therefore, the present review is focussed on the characterization of kinesin, besides providing a short overview on the other cytoskeletal motor proteins (table).

Properties of cytoskeleton-associated linear motors

Exponent	Rail	Supply	Step size [nm]	Velocity [μm/s]	Force [pN]	Driving direction
Conventional kinesin	MT	ATP	8	0.6-0.8	4–8	Plus
Axonemal dynein (22S)	MT	ATP	8	1.0-5.0	4.7	Minus
Cytoplasmic dynein	MT	ATP		0.3	2–3	Minus
Myosin II	MF	ATP	4–15	0.2–8.0	3.3	Plus
Myosin V	MF	ATP	20–36	0.4–0.6	~3	Plus
Myosin VI*	MF	ATP	30	0.3	2.8	Minus

Note. This table does not cover the broad variety of values determined for the numerous multiple forms of motor proteins under diverse conditions. MT — microtubule; MF — microfilament.

Myosins and dyneins

Myosin II. Myosin II is known to be the engine for skeletal, cardiac, and smooth muscle contraction. Native myosin II consists of two heavy chains (200 kDa) and four light chains (two chains with 20 kDa, the other ones with 15 kDa and 25 kDa), resulting in a molecular mass of approximately 500 kDa. The heavy chains can be characterized as large asymmetric molecules with two globular heads and an about 150-nm long tail. A characteristic feature of myosin II is its ability to self-assemble into bipolar, so-called thick, filaments, which constitute the sarcomeres together with the thin filaments (microfilaments), formed from alpha actin. Muscle contraction is a result of sarcomere shortening based on sliding between the thick and thin filaments using the energy released by ATP hydrolysis [12].

Myosin II moves in 4- to 15-nm steps along the microfilaments at velocities between 0.2 and 8 μm/s (see [13]). It is a non-processive motor undergoing one catalytic cycle per diffusional encounter with the microfilament track [14]. Its ATPase was found to be activated by actin filaments [15].

The mechanism of myosin force generation can be studied by *in vitro* motility assays. So, it has been shown that actin filaments slide on glass or mica surfaces, covered by heavy myosin chains (HMM). Movement can be visualized directly by fluorescence labelling of the actin or indirectly by binding of actin filaments to polystyrene beads [16]. The second approach of a motility assay has the advantage that it can be combined with an optical trapping technique. Using this technique, the maximum force associated with the movement was found to be 3.3 pN [17]. The group of Yanagida introduced a micro needle manipulation technique to measure the unitary steps and forces of myosin movement. The authors showed that the myosin head generates a force of even 5.9 + 0.8 pN at peak (2.1 pN on average) over the whole ATPase cycle [18].

Myosin V and myosin VI. These motor proteins are myosin variants which interact with the microfilaments of *non-muscle* cells, known to consist of beta- and gamma-actin. They are mainly involved in organelle transport. Myosin VI was reported to be implicated also in stereo cilia functioning [19].

Like skeletal myosin, myosin V molecules have two heads. The myosin-V heads are attached to an approximately 30-nm stalk that ends in a globular region [20]. Myosin V has actin-activated magnesium-ATPase activity, does not form thick filaments, and is a barbed (plus-) end-directed motor capable to move microfilaments at velocities of up to 0.4 μm/s [20]. Moreover, it was shown to be able to transport artificial beads (e.g., silica beads) along actin filaments at velocities between 0.36 and 0.64 μm/s [21].

Motility assays combining filament gliding and optical trapping to study single molecules indicated that myosin V is a highly efficient processive motor [22, 23]. It is known that the actin subunits within the microfilaments are arranged helically with a repeat of 36 nm (see [24]). The question arises whether in this case a single two-headed myosin molecule can transport a cargo by walking straight. Unlike muscle myosin whose heads are only about 16 nm long, myosin V has heads of about 31 nm enabling to span the 36-nm repeat and consequently a straight transport along the microfilaments [24, 25].

Contrary to all the other 17 known classes of myosins (see [26]), myosin VI moves to the minus end [19, 26, 27]. Using chimeric myosins that comprise the motor domain and the lever arm domain from a myosin type that moves in the opposite direction, it could be shown that the motor core domain, but neither the large insert nor the converter domain, determines the direction of myosin motility [26].

Axonemal dyneins. These dyneins are motors distributed along the surface of microtubule doublets in flagella and cilia. Multiple forms of dynein have been isolated from axonemes, even in a single species [28]. In the electron microscope, the outer dynein molecules of the axoneme reveal a bouquet-like structure consisting of a branched stalk with three globular heads of about 10 nm diameter each and a molecular mass of 450 kDa. Unlike most of the other cytoskeletal motor proteins, this dynein lacks long coiled coil regions. In the presence of ATP, axonemal dynein walks along the surface of microtubules towards their minus-ends [29]. The velocity was found to be variable between 1 and 5 μm/s [29] and seems to depend on microtubule length [30]. Microtubules stimulate the ATPase activity of the 22 S dynein [31]. During force generation, 22S dynein revealed a stepwise displacement of nearly 8 nm [28], corresponding to the tubulin dimer length. Axonemal dynein was suggested to be able to switch over from a processive mode to a non-processive mode [28].

Cytoplasmic dyneins. Being the cytoplasmic counterpart to the axonemal dyneins, cytoplasmic dynein (or MAP 1C) is responsible for the retrograde organelle transport. Besides, there are forms of cytoplasmic dynein which were suggested to participate in maintaining the spatial pattern of the interphase microtubule network [32]. In this context, it should be mentioned that cytoplasmic dynein is able to induce the formation of radial microtubule arrays through a process of self-organization [33] and to crossbridge microtubules into bundles [34].

Each molecule of cytoplasmic dynein has two globular heads of about 12 nm in diameter, containing the microtubule-activated ATPase and the microtubule-binding site. With its total length of approximately 45 nm, it is remarkably shorter than conventional kinesin. Moving to the minus end of microtubules [35, 36], cytoplasmic dynein has been suggested not to be a processive motor protein by itself [37]. *In vitro* motility assays with latex beads demonstrated that, unlike conventional kinesin, cytoplasmic dynein changes relatively often (on average once per 0.2 μm) from one microtubule protofilament to the adjacent one [38, 39]. The processivity of cytoplasmic dynein can be improved by the action of special protein factors, *e.g.*, dynactin [37] or by partial proteolytical removal of the negatively charged C-terminal tails of the tubulin dimers [40]. Under both *in vitro* [40] *in vivo* [41] conditions, this dynein moves at velocities up to about 1 μm/s. The force that a single cytoplasmic dynein exerts *in vivo* was found to be 1.1 pN [41].

Kinesin – biochemical and biophysical properties

Like the dyneins, kinesins are obligate microtubule-associated motor proteins which transport different cytoplasmic organelles and vesicles, macromolecular complexes [42], but also, chromosomes to defined destinations in eukaryotic cells. Walking along the microtubule rails together with the cargo, they convert the chemical energy of nucleoside triphosphates (preferentially from ATP) into mechanical energy.

Within the last decade, numerous kinesin isoforms and related proteins, sharing a highly conserved motor domain of 340–350 amino acids [43, 44], have been described for animal and plant cells as well as for lower eukaryotic organisms [45–47]. A prominent member of the kinesin superfamily, comprising more than 100 subspecies [43], is the conventional kinesin which essentially contributes to anterograde vesicle in neuronal cells. The kinesin ATPase is strongly promoted by microtubules [48–50]. Cargo binding has been shown to be also able to affect the functional activity of kinesins [42, 51].

Native conventional kinesin consists of two heavy (120 to 130 kDa) and two light chains (60 to 70 kDa) resulting in a molecular mass of about 400 kDa [48, 52–54]. Each heavy chain contains a N-terminal globular motor domain with both a microtubule-binding site and an ATPase-active centre, a stalk region, which is responsible for heavy chain dimerization, and a C-terminal globular tail domain, implicated presumably together with the stalk in cargo binding. The light chains are believed to be involved in the kinesin binding to organelles, are not essential for motility generation [55], and seem to have a regulatory function [56]. In the electron microscope, kinesin molecules appear as thin flexible filaments (about 80 nm in length) with two globular heads (about 6 nm in diameter) and a stalk with spliced ends

[57]. The molecular structure of the kinesin molecule functionally depends on the actual state of the mechanochemical cycle, i.e., it is determined by the phases of microtubule binding, ATP binding, ATP hydrolysis, and the release of the hydrolysis products ADP and P_i [58].

The complete amino acid sequences of different kinesins are known. Motility-active recombinant heavy chains of kinesin and additionally different heavy chain domains can be expressed in bacterial hosts [57] and animal cell cultures [59], providing the opportunity to prepare kinesin on a milligram scale in defined quality.

Kinesin generates motility by walking step by step and hand-over-hand along the surface of microtubules [60], functioning as rails. Microtubules are hollow-cylindrical protein polymers of inner and outer diameters of about 14 and 25 nm, respectively, and lengths up to a few tens of μm (see *e.g.* [61]). They are composed of so-called tubulin protofilaments which can be described as chain-like association products of alpha and beta tubulin. Because of the dimeric character of the tubulin and the strict alternation of alpha and beta, one end of the protofilament is terminated by an alpha subunit and the opposite one by a beta subunit. This provides the protofilament a certain kind of polarity. The microtubule wall is built up by protofilaments laterally associated with same polarity. Consequently one microtubule end exposes alpha subunits (minus end) and the opposite end beta subunits (plus end) only [62]. Under steady state conditions, at the plus end more dimers are added than lost and reversely, at the minus end more dimers are released than new ones re-associate.

The direction of kinesin movement depends on both microtubule polarity and the intrinsic molecular structure of kinesin [63–65]. Conventional kinesin is known to be a plus-end directed motor. There are a few other kinesin-like motors like the nonclaret disjunctional protein (ncd), required for chromosome distribution, that move to the minus end [66]. Dimeric constructs in which the motor domains are located either at the N- or the C-terminal end of the heavy chain were shown to move towards microtubule plus or minus ends, respectively [67]. In addition, the directionality of kinesin seems to be also dependent on neck/motor core interactions [68].

A characteristic feature of the two-headed conventional kinesin is its processivity, i.e., single kinesin molecules move along microtubules without dissociating [69–71]. Both heads translocate along one and the same protofilament [72, 73] by turns in 16-nm steps, resulting in 8-nm centre-of-mass shifts [58, 74]. One of the heads remains bound to the microtubule when the second one moves [57, 62]. Using an assay that resolves nanometre displacements of single kinesin molecules with microsecond accuracy, Nishiyama et al. [75] demonstrated that the 8-nm shifts can be resolved into fast and slow sub steps, each corresponding to a displacement of approximately 4 nm. Recently, it has been shown that also one-headed members of the kinesin superfamily, *e.g.*, KIF1A, can realize movement in a processive fashion. This was explained by the existence of two microtubule-binding motifs in one head [76]. At near-zero loads, kinesin molecules hydrolyse one ATP molecule per 8-nm shift [76, 77].

Intracellular movement and transport can be simulated *in vitro* using purified kinesin and taxol-stabilized microtubules in a so-called microtubule gliding assay, in which microtubules move across a kinesin coated surface [62, 78]. Alternatively, another motility assay to study the molecular mechanisms of kinesin functioning can be used in which kinesin-coated polymer beads translocate along microtubules immobilized to glass [37, 70].

Using conventional kinesins, the velocities measured for gliding microtubules in vitro were about 0.4–0.9 μm/s [79]. For comparison, a kinesin from the fungus *Neurospora* was found to walk significantly faster (up to 3.8 μm/s [79]). In contrast, the mitotic kinesin Eg5 moves at about 0.06 μm/s, only [80]. For conventional kinesin it was shown that microtubule gliding can be accelerated by increasing the Mg^{2+} concentration at a constant ATP concentration [72, 81] and by temperature elevation [82]. At high temperature velocities up to 3.7 μm/s were measured [83]. Moreover, it could be demonstrated that, within certain intervals, the velocity of microtubule movement across kinesin-coated glass surfaces is reduced with increasing density of the bound kinesin [81, 84]. The conventional kinesin was found to work at temperatures between 6 and 40 °C, higher temperature causes irreversible denaturation [82]. Using temperature pulse microscopy, it has been recently shown that under favourable conditions kinesin can work up to 30 min even at temperatures up to 50 °C [83].

Several approaches have been used to measure forces generated by *single* kinesin molecules, including movement against a viscous load [84], a cen-

trifuge microscope-based motility assay [85], optical trapping interferometry [86, 87], and optical microscopy equipped with optical tweezers [88]. The forces determined were within the range between 4 and 8 pN.

So far, the molecular mechanisms of force generation realized by *more than one* kinesin molecule contributing to the transport of one and the same cargo are not completely understood. It is not known whether kinesin molecules collectively involved in cargo transport synchronize themselves or work non-synchronously and whether special cellular factors are required for synchronization.

Kinesin-based nanoactuatoric developments

Preconditions to construct kinesin-based nanoactuators. Several groups have started to fit the kinesin-microtubule machinery for the development of miniaturized technical devices that realize transport with nanometre precision. However, before this bio motor can be really exploited numerous methodological and technical problems have to be solved. Complex studies should be performed to learn *e.g.* to regulate the velocity and to increase the life time of the kinesin motor, to find out technically relevant materials enabling its work in cell-free environment, or to control the direction of force generation. To work out a basic configuration of a kinesin-driven motor device incorporated into a spatially limited system (chambers or pipes with sub micrometre heights or diameters, respectively) the minimal clearance still allowing kinesin force generation has to be determined. First approaches have been published contributing to the solution of these problems [89–93, 98].

Isopolar and parallel immobilization of the microtubule rails. A critical point in the development of a cell-free kinesin-based machinery is the predetermination and control of transport direction. Turner et al. [89] immobilized microtubules in parallel fashion on lithographically patterned silane surfaces using a flow field. The microtubule tracks prepared in this way were shown to be suitable rails for the transport of kinesin-coated beads. Limberis and Stewart [91] demonstrated that also silicon microchips can be moved by kinesin across flow field-aligned microtubules. Alternatively, Dennis et al. [90] used highly oriented polymer films coated by kinesin to drive microtubules in nearly straight lines.

Both the flow field-based approach and the immobilization on lithographically patterned surfaces yielded arrays of microtubules which were aligned parallel in geometrical sense, but not with equal polarity. When arrays of microtubule of opposite polarity were used as rails to transport especially a large cargo binding to more than one microtubule the elementary forces generated along adjacent microtubules may act in different direction and neutralize each other more or less completely. Therefore, such arrays seem not to be well suited for an efficient cargo transport and consequently cannot find broad application in nanotechnology.

Recently, Hiratsuka et al. [94] produced linear micro lithographic tracks on glass surfaces supplemented with arrowhead patterns, allowing moving the kinesin in one direction, only. Alternatively, microtubules were demonstrated to align in parallel and isopolar fashion during gliding across kinesin-coated glass surfaces by application of flow fields [92]. However, when the flow was abolished the microtubules immediately started to move again in directionally uncontrolled manner. It is known that microtubules treated by glutaraldehyde retained their potency to serve as tracks for kinesin movement [95]. Using glutaraldehyde for immobilization, stable isopolar microtubule arrays could be prepared (Fig. 1) which were proved to be able to transport in the absence of the flow also large kinesin-coated particles in a predetermined direction [93] (Fig. 2).

Track lengths and ATP supply. A further important parameter in fitting the kinesin-based motor system for nanotechnological applications is the track length. Under conditions of high kinesin density, gliding microtubules were shown to cover distances much greater than their length [96]. Dependent on the tubulin concentration, the temperature gradient during assembly, and the incubation time at polymerization temperature, the microtubules formed *in vitro* are about 5 to 40 μm long. To convey a cargo along microtubules over distances significantly exceeding their length, an efficient transition of the cargo from one microtubule to an adjacent one should be enabled. This requirement can be fulfilled by preparation of surfaces with densely packed microtubule arrays. It was shown that at high microtubule surface density micrometre-sized cargoes can be transported over distances of some millimetres into a desired direction [93]. Such an approach seems to be not only of basic interest for special future developments in the

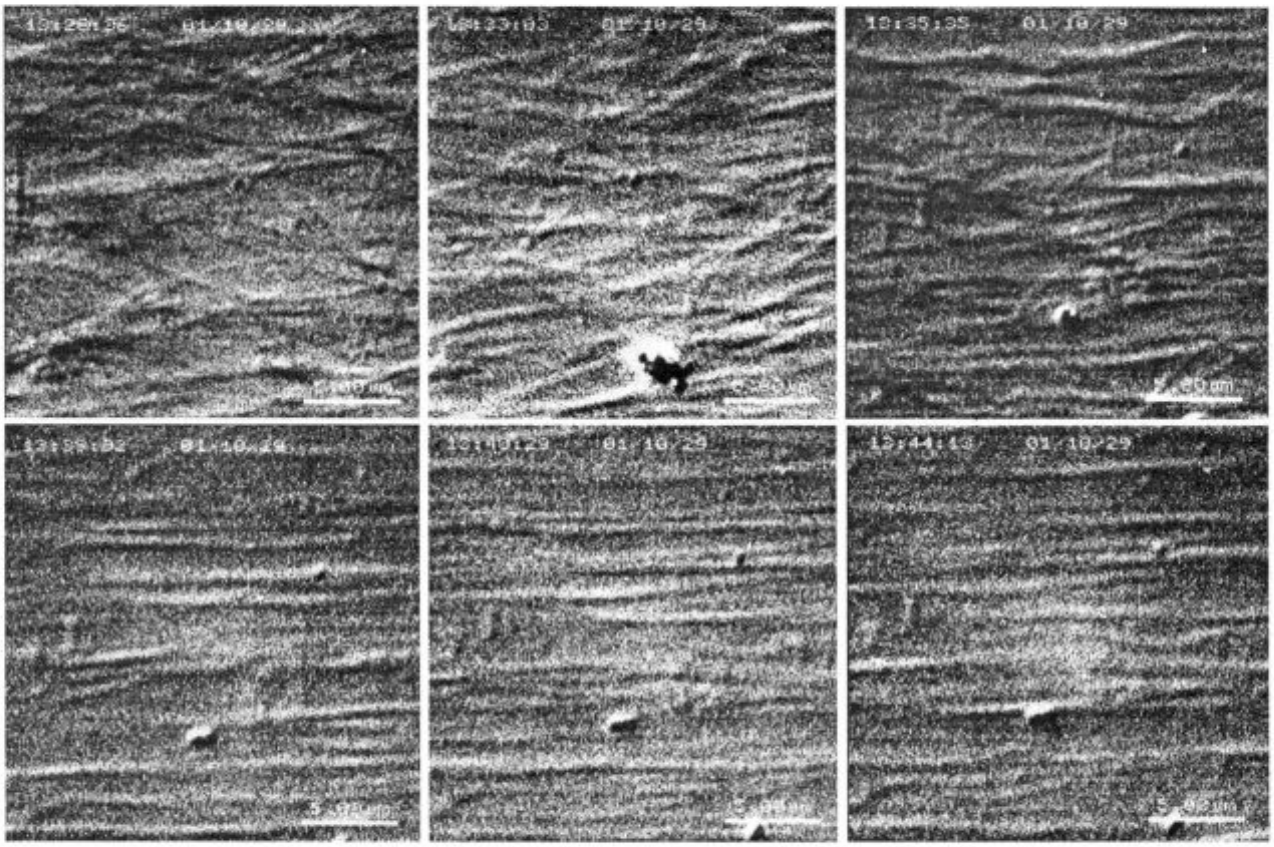

Fig. 1. Flow-field induced orientation of gliding microtubules, demonstrated by video-enhanced DIC microscopy. 1st image: stochastic gliding of the microtubules. 2nd to 5th: consecutive isopolar alignment of microtubules by flow field. The leading (minus) end of the microtubules is turned in flow direction. 6th image: isopolar microtubules stabilized by glutaraldehyde. Video-enhanced contrast microscopic images. The bars represent 5 μm. This figure was kindly provided by Janina Beeg, Jena

field of nanotechnology, but is believed to be also a valuable tool for cell biology usable to determine the directionality of non-characterized microtubule-associated motor proteins.

Kinesin-driven microtubule gliding as well as the translocation of kinesin-coated beads is usually performed at ATP concentrations between 0.5 mM and 10 mM, corresponding to $0.3 \cdot 10^{15}$ and $6 \cdot 10^{15}$ ATP molecules within a chamber of 1 μl volume. Each kinesin molecule consumes one molecule ATP to translocate a 200-nm silica bead over a distance of 8 nm (97). This means that with 0.5 mM ATP in a 1-μl volume a bead can be theoretically moved over a distance of $2.4 \cdot 10^{6}$ m. An alternative calculation gives $4.8 \cdot 10^{8}$ beads that can be transported over a track length of 5 mm. However, it should be mentioned that the adenosine diphosphate (ADP) released during the mechanochemical cycle of kinesin exerts an inhibitory action. We observed a linear decrease of the velocity of microtubule gliding across a kinesin-coated surface with decreasing ATP concentration and corresponding ADP accumulation (Fig. 3). To overcome this problem, an ATP-regenerating system (see [99]) might be introduced.

Basic configuration of a kinesin-based motor and possible applications. Nano-scaled devices using the functional activity and structural features of biological materials are becoming more and more reality. Currently, great efforts are being made to elaborate biological concepts applicable for the development of *e.g.*, chip-based nanosensors, opto nanobiosystems, nano-scaled information-processing devices, or nano-scaled machinery implementing the motor proteins. Innovative improvements in the properties of the biological materials are believed to contribute to the construction of controllable, nanometre-scaled,

lower cost and higher performance nano devices, machines or robots.

The nano biotechnological development of linear motor devices composed of stabilized microtubules and conventional kinesin, isolated from brain or produced by recombinant techniques, has reached a remarkable state [93, 94, 98]. The isopolar microtubule alignment allows conveying not only nanometre-sized cargoes but also larger ones (up to particles of about 20 × 20 × 5 μm) over, in nanotechnological meaning, practically unlimited distances (> 1 mm). The cargoes or the supports used to carry the biomolecular force-generating unit can be made from glass, quartz, carbon, gold, polystyrene, and even from the micro technologically most important material, the silicon (own results). All these materials investigated do not have any significant influence on the transport parameters. As a practically unlimited energy source for a nano-scaled motor device, ATP can be used. The velocity of the kinesin motor can be tuned between 0 and 2 μm/s by changing the environment variables, *e.g.* temperature [82] and cofactor concentrations [81], or by adding polyhydroxy compounds like glycerol [100]. The kinesin motor works between temperatures from 6 to 40 °C [81], under special conditions even to 50 °C [83].

One important point in the development of motor-protein based nanoactuators concerns the maintenance of the motor activity over several hours. At sufficient ATP supply, microtubule gliding in a closed glass chamber can be observed at least up to 3 h [81]. When kinesin-coated particles were applied to isopolar microtubule arrays, they were also observed to move for 2 or 3 h, thereafter these arrays can be used again for the transport of fresh motor protein-coated particles. The pre-assembled force-generating system can be stored in frozen state (own observations).

The surface of such a device must not be ideally planar, a roughness and dust particles of height differences up to 280 nm were shown to be tolerated [92]. The minimum working height, the clearance, was determined to be about 100 nm [92]. This has to be considered in construction of sub micrometre-scaled planar devices or transport pipes.

The long-term impact of the involvement of motor proteins in nanotechnological developments is difficult to predict. From present view, potential realizable applications of cell-free motility nano systems (motors and actuators) can be:

— switchable linear motor devices and molecular machines working with nanometre precision which might be applied for a directionally controlled displacement of objects or for a specific and directed substance transport over nm, μm, or mm-distances. Such devices can contribute to *e.g.*, the assembling and positioning of electronic circuits or sensors, to nanostructure assembly, and nanostructure fabrication;

— transport of chemical components or catalysts into or out of micro reactors, used for *e.g.*, enhancing and controlling reactions;

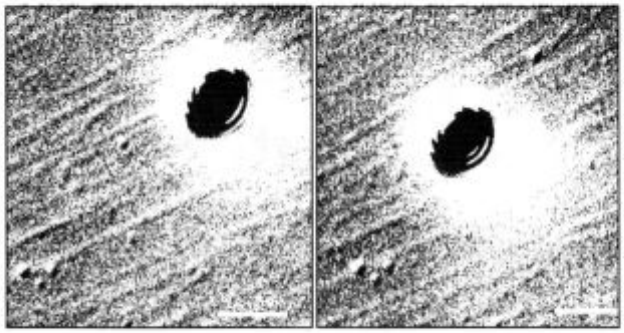

Fig. 2. Translocation of a spherical paramagnetic bead (2.8 μm in diameter) along isopolar microtubules arrays. The microtubules appear as filamentous structures in the background. The bars represent 5 μm. This figure was kindly provided by Janina Beeg, Jena

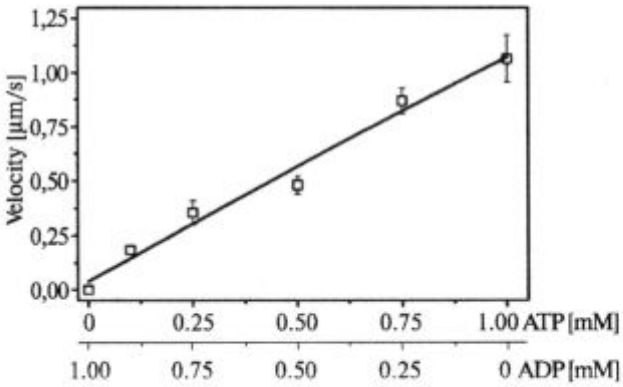

Fig. 3. Effect of complementary ATP and ADP concentrations on the velocity of microtubule gliding across a kinesin-coated surface. Motility mix: 0.15 mg/ml kinesin purified from brain [48], taxol-stabilized microtubules (corresponding to 0.04 mg/ml tubulin), 50 mM imidazole pH 6.8, 1.0 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM dithiothreitol, 100 mM NaCl, 20 μM taxol, ATP and ADP sodium salts as indicated. The motility mix was applied onto glass slides, pretreated with a solution of 5 mg/ml bovine serum albumin in the same buffer. Gliding velocity was measured using the speed function of the ARGUS 20-image processing software and video-enhanced contrast microscopy

— development of intracellular sensors for diagnostic purposes;

— delivery systems to convey drugs to pathological targets;

— screening systems for developing novel therapeutical drugs targeting cellular movement;

— autonomously working power units to drive implantable sensors with cell-own ATP;

— cell biological tools to study functional or regulatory mechanisms of motor protein-based force generation.

Outlook

Taken together, the design and control of kinesin-based nanoactuatoric devices has been advanced, but several serious problems remain to be solved until such a device can be really used as an integral part of a complex nanoactuatoric machine or robot.

One main point that has not been addressed in future is the regulation of starting and stopping force generation under cell-free conditions in millisecond ranges, *i.e.* with nanometre precision. Further unsolved problems concern the biological activity of kinesin motor, the maximum load mass that can be moved by a collective of kinesin molecules, and the synchronization of a great number of kinesin molecules contributing to the transport of one and the same cargo.

Further cell biological, biochemical, and biophysical work combined with experiments of technical performance has to be done in future to realize the ambitious tasks nanoactuatoric application of bio motors.

This work has been supported by the Deutsche Forschungsgemeinschaft, grant numbers INK 22/Bl-1/A5 and Un82/5-1.

РЕЗЮМЕ. Эукариотические организмы синтезируют разнообразные двигательные белки, преобразующие химическую энергию в механическую. Среди них обнаружены как ротационные (например, АТР-синтаза), так и линейные двигатели. Линейные двигатели охватывают высокоспециализированные белки, двигающиеся вдоль филаментов нуклеиновых кислот (например, РНК-полимераза) или цитоскелета. В настоящей статье представлен краткий обзор двигателей, связанных с цитоскелетом (миозины, динеины и кинезины), и подводятся итоги исследований, вносящих вклад в разработку базовой конфигурации для создания приводимого в движение кинезином двигательного устройства, пригодного, например, для контролированного перемещения объектов или специальных веществ на расстояния порядка миллиметра с точностью до нанометров.

РЕЗЮМЕ. Еукаріотичні організми синтезують різноманітні моторні білки, що перетворюють хімічну енергію у механічну. Серед них виявлено як ротаційні (наприклад, АТР-синтаза), так і лінійні двигуни. Лінійні двигуни охоплюють високоспеціалізовані білки, що рухаються вздовж філаментів нуклеїнових кислот (наприклад, РНК-полімераза) чи цитоскелета. У даній статті наведено короткий огляд двигунів, пов'язаних із цитоскелетом (міозини, динеїни і кінезини), та підсумовуються результати досліджень, що роблять внесок у розробку базової конфігурації для створення пристрою, який приводиться в рух за допомогою кінезина, придатного, наприклад, для контрольованого переміщення об'єктів чи спеціальних речовин на відстані порядку міліметра з точністю до нанометрів.

REFERENCES

1. *Berg H.C.* Torque generation by the flagellar rotary motor // Biophys. J. — 1995. — **68.** — P. 163S–167S.
2. *Berg H.C.* Constraints on models for the flagellar rotary motor // Philos. Trans. R. Soc. Lond. B Biol. Sci. — 2000. — **355.** — P. 491–501.
3. *Engelbrecht S., Junge W.* ATP synthase: a tentative structural model // FEBS Lett. — 1997. — **414.** — P. 485–491.
4. *Cross R.L.* The rotary binding change mechanism of ATP synthases // Biochim. Biophys. Acta. — 2000. — **1458.** — P. 270–275.
5. *Montemagno C., Bachand G.* Constructing nanomechanical devices powered by biomolecular motors // Nanotechnol. — 1999. — **10.** — P. 225–231.
6. *Gelles J., Landick R.* RNA polymerase as a molecular motor // Cell. — 1998. — **93.** — P. 13–16.
7. *Wang M.D.* Manipulation of single molecules in biology // Curr. Opin. Biotechnol. — 1999. — **10.** — P. 81–86.
8. *Kabata H., Kurosawa O., Arai I., Washizu M., Margarson S.A., Glass R.E., Shimamoto N.* Visualization of single molecule dynamics of RNA polymerase sliding along DNA // Science. — 1993. — **262.** — P. 1561–1563.
9. *Kabata H., Shimamoto N.* Single-molecule dynamics of protein interplaying with nonspecific DNA // Biophysics. — 1995. — **202.** — P. 277–279.
10. *Yin H., Wang M.D., Svoboda K., Landick R., Block S.M., Gelles J.* Transcription against an applied force // Science. — 1995. — **270.** — P. 1653–1657.
11. *Wang M.D., Schnitzer M.J., Yin H., Landick R., Gelles J., Block S.M.* Force and velocity measured for single molecules of RNA polymerase // Science. — 1998. — **282.** — P. 902–907.
12. *Huxley A.F.* Mechanics and models of the myosin motor // Philos. Trans. R. Soc. Lond. B. Biol. Sci. — 2000. — **355.** — P. 433–440.
13. *Howard J.* Molecular motors: structural adaptations to cellular functions // Nature. — 1997. — **389.** — P. 561–567.
14. *Mehta A.D., Rief M., Spudich J.A., Smith D.A., Simmons R.M.* Single-molecule biomechanics with optical methods // Science. — 1999. — **283.** — P. 1689–1695.
15. *Koffer A, Sleep J.* Activation of myosin ATPase by actin isolated from cultured BHK cells and the effect of gelsolin // J. Muscle Res. Cell Motil. — 1987. — **8.** — P. 541–547.

16. *Kawai M., Kawaguchi K., Saito M., Ishiwata S.* Temperature change does not affect force between single actin filaments and HMM from rabbit muscles // Biophys. J. — 2000. — **78**. — P. 3112–3119.
17. *Miyata H., Yoshikawa H., Hakozaki H., Suzuki N., Furuno T., Ikegami A., Kinosita Jr. K., Nishizaka T., Ishiwata S.* Mechanical measurements of single actomyosin motor force // Biophys. J. — 1995. — **68**. — P. 286S–290S.
18. *Ishijima A., Kojima H., Higuchi H., Harada Y., Funatsu T., Yanagida T.* Multiple- and single-molecule analysis of the actomyosin motor by nanometer-piconewton manipulation with a microneedle: unitary steps and forces // Biophys. J. — 1996. — **70**. — P. 383–400.
19. *Rock R.S., Rice S.E., Wells A.L., Purcell T.J., Spudich J.A., Sweeney H.L.* Myosin VI is a processive motor with a large step size // Proc. Nat. Acad. Sci. USA. — 2001. — **98**. — P. 13655–13659.
20. *Cheney R.E., O'Shea M.K., Heuser J.E., Coelho M.V., Wolenski J.S., Espreafico E.M., Forscher P., Larson R.E., Mooseker M.S.* Brain myosin-V is a two-headed unconventional myosin with motor activity // Cell. — 1993. — **75**. — P. 13–23.
21. *Wolenski J.S., Cheney R.E., Mooseker M.S., Forscher P.* In vitro motility of immunoadsorbed brain myosin-V using a Limulus acrosomal process and optical tweezer-based assay // J. Cell Sci. — 1995. — **108**. — P. 1489–1496.
22. *Moore J.R., Krementsova E.B., Trybus K.M., Warshaw D.M.* Myosin V exhibits a high duty cycle and large unitary displacement // J. Cell Biol. — 2001. — **155**. — P. 625–636.
23. *Rock R.S., Rief M., Mehta A.D., Spudich J.A.* In vitro assays of processive myosin motors // Methods. — 2000. — **22**. — P. 373–381.
24. *Walker M.L., Burgess S.A., Sellers J.R., Wang F., Hammer III J.A., Trinick J., Knight P.J.* Two-headed binding of a processive myosin to F-actin // Nature. — 2000. — **405**. — P. 804–807.
25. *Rief M., Rock R.S., Mehta A.D., Mooseker M.S., Cheney R.E., Spudich J.A.* Myosin-V stepping kinetics: a molecular model for processivity // Proc. Nat. Acad. Sci. USA. — 2000. — **97**. — P. 9357–9359.
26. *Homma K., Yoshimura M., Saito J., Ikebe R., Ikebe M.* The core of the motor domain determines the direction of myosin movement // Nature. — 2001. — **412**. — P. 831–834.
27. *Wells A.L., Lin A.W., Chen L.Q., Safer D., Cain S.M., Hasson T., Carragher B.O., Milligan R.A., Sweeney H.L.* Myosin VI is an actin-based motor that moves backwards // Nature. — 1999. — **401**. — P. 505–508.
28. *Hirakawa E., Higuchi H., Toyoshima Y.Y.* Processive movement of single 22S dynein molecules occurs only at low ATP concentrations // Proc. Nat. Acad. Sci. USA. — 2000. — **97**. — P. 2533–2537.
29. *Yamada A., Yamaga T., Sakakibara H., Nakayama H., Oiwa K.* Unidirectional movement of fluorescent microtubules on rows of dynein arms of disintegrated axonemes // J. Cell Sci. — 1998. — **111**. — P. 93–98.
30. *Hamasaki T., Holwill M.E., Barkalow K., Satir P.* Mechanochemical aspects of axonemal dynein activity studied by in vitro microtubule translocation // Biophys. J. — 1995. — **69**. — P. 2569–2579.
31. *Omoto C.K., Johnson K.A.* Activation of the dynein adenosinetriphosphatase by microtubules // Mol. Biol. Cell. — 1996. — 7. — P. 935–948.
32. *Koonce M.P., Samso M.* Overexpression of cytoplasmic dynein's globular head causes a collapse of the interphase microtubule network in Dictyostelium // Mol. Biol. Cell. — 1996. — **7**. — P. 935–948.
33. *Vorobjev I., Malikov V., Rodionov V.* Self-organization of a radial microtubule array by dynein-dependent nucleation of microtubules // Proc. Nat. Acad. Sci. USA. — 2001. — **98**. — P. 10160–10165.
34. *Amos L.A.* Brain dynein crossbridges microtubules into bundles // J. Cell Sci. — 1989. — **93**. — P. 19–28.
35. *Paschal B.M., Shpetner H.S., Vallee R.B.* MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties // J. Cell Biol. — 1987. — **105**. — P. 1273–1282.
36. *Shimizu T., Toyoshima Y.Y., Edamatsu M., Vale R.D.* Comparison of the motile and enzymatic properties of two microtubule minus-end-directed motors, ncd and cytoplasmic dynein // Biochem. — 1995. — **34**. — P. 1575–1582.
37. *King S.J., Schroer T.A.* Dynactin increases the processivity of the cytoplasmic dynein motor // Nat. Cell Biol. — 2000. — **2**. — P. 20–24.
38. *Wang Z.H., Khan S., Sheetz M.P.* Single cytoplasmic dynein molecule movements: Characterization and comparison with kinesin // Biophys. J. — 1995. — **69**. — P. 2011–2023.
39. *Kuo S.C., Gelles J., Steuer E., Sheetz M.P.* A model for kinesin movement from nanometer-level movements of kinesin and cytoplasmic dynein and force measurements // J. Cell Sci. Suppl. — 1991. — **14**. — P. 135–138.
40. *Wang Z., Sheetz M.P.* The C-terminus of tubulin increases cytoplasmic dynein and kinesin processivity // Biophys. J. — 2000. — **78**. — P. 1955–1964.
41. *Gross S.P., Welte M.A., Block S.M., Wieschaus E.F.* Dynein-mediated cargo transport in vivo. A switch controls travel distance // J. Cell Biol. — 2000. — **148**. — P. 945–956.
42. *Terada S., Hirokawa N.* Moving on to the cargo problem of microtubule-dependent motors in neurons // Curr. Opin. Neurobiol. — 2000. — **10**. — P. 566–573.
43. *Sakowicz R., Berdelis M.S., Ray K., Blackburn C.L., Hopmann C., Faulkner D.J., Goldstein L.S.* A marine natural product inhibitor of kinesin motors // Science. — 1998. — **280**. — P. 292–295.
44. *Stewart R.J., Thaler J.P., Goldstein L.S.* Direction of microtubule movement is an intrinsic property of the motor domains of kinesin heavy chain and Drosophila ncd protein // Proc. Nat. Acad. Sci. USA. — 1993. — **90**. — P. 5209–5213.
45. *Vale R.D., Fletterick R.J.* The design plan of kinesin motors // Ann. Rev. Cell Dev. Biol. — 1997. — **13**. — P. 745–777.
46. *Hirokawa N.* Kinesin and dynein superfamily proteins and the mechanism of organelle transport // Science. — 1998. — **279**. — P. 519–526.
47. *Miki H., Setou M., Kaneshiro K., Hirokawa N.* All kinesin superfamily protein, KIF, genes in mouse and human // Proc. Nat. Acad. Sci. USA. — 2001. — **98**. — P. 7004–7011.

48. *Kuznetsov S.A., Gelfand V.I.* Bovine brain kinesin is a microtubule-activated ATPase // Proc. Nat. Acad. Sci. USA. — 1986. — **83**. — P. 8530–8534.
49. *Huang T.G., Hackney D.D.* Drosophila kinesin minimal motor domain expressed in Escherichia coli. Purification and kinetic characterization // J. Biol. Chem. — 1994. — **269**. — P. 16493–16501.
50. *Johnson K.A., Gilbert S.P.* Pathway of the microtubule-kinesin ATPase // Biophys. J. — 1995. — **68**. — P. 173S–179S.
51. *Friedman D.S., Vale R.D.* Single-molecule analysis of kinesin motility reveals regulation by the cargo-binding tail domain // Nat. Cell Biol. — 1999. — **1**. — P. 293–297.
52. *Bloom G.S., Wagner M.C., Pfister K.K., Brady S.T.* Native structure and physical properties of bovine brain kinesin and identification of the ATP-binding subunit polypeptide // Biochem. — 1988. — **27**. — P. 3409–3416.
53. *Scholey J.M., Heuser J., Yang J.T., Goldstein L.S.* Identification of globular mechanochemical heads of kinesin // Nature. — 1989. — **338**. — P. 355–357.
54. *Kuznetsov S.A., Gelfand V.I.* Purification of kinesin from the brain // Meth. Mol. Biol. — 2001. — **164**. — P. 1–7.
55. *Howard J.* Molecular motors: structural adaptations to cellular functions // Nature. — 1997. — **389**. — P. 561–567.
56. *Verhey K.J., Lizotte D.L., Abramson T., Barenboim L., Schnapp B.J., Rapoport T.A.* Light chain-dependent regulation of kinesin's interaction with microtubules // J. Cell Biol. — 1998. — 143. — P. 1053–1066.
57. *Kozielski F., Arnal I., Wade R.H.* A model of the microtubule-kinesin complex based on electron cryomicroscopy and X-ray crystallography // Curr. Biol. — 1998. — **8**. — P. 191–198.
58. *Mandelkow E., Johnson K.A.* The structural and mechanochemical cycle of kinesin // Trends Biochem. Sci. — 1998. — **23**. — P. 429–433.
59. *Navone F., Niclas J., Hom-Booher N., Sparks L., Bernstein H.D., McCaffrey G., Vale R.D.* Cloning and expression of a human kinesin heavy chain gene: interaction of the COOH-terminal domain with cytoplasmic microtubules in transfected CV-1 cells // J. Cell Biol. — 1992. — **117**. — P. 1263–1275.
60. *Kawaguchi K., Ishiwata S.* Nucleotide-dependent single- to double-headed binding of kinesin // Science. — 2001. — **291**. — P. 667–669.
61. *Unger E., Buhm K.J., Vater W.* Structural diversity and dynamics of microtubules and polymorphic tubulin assemblies // Electron Microsc. Rev. — 1990. — **3**. — P. 355–395.
62. *Nogales E., Wolf S.G., Downing K.H.* Structure of the alpha beta tubulin dimer by electron crystallography // Nature. — 1998. — **391**. — P. 199–203.
63. *Henningsen U., Schliwa M.* Reversal in the direction of movement of a molecular motor // Nature. — 1997. — **389**. — P. 93–96.
64. *Woehlke G., Schliwa M.* Directional motility of kinesin motor proteins // Biochim. Biophys. Acta. — 2000. — **1496**. — P. 117–127.
65. *Cross R.A.* Motor proteins. Directing direction // Nature. — 2000. — **406**. — P. 839–840.
66. *Foster K.A., Mackey A.T., Gilbert S.P.* A mechanistic model for Ncd directionality // J. Biol. Chem. — 2001. — **276**. — P. 19259–19266.
67. *Wade R.H., Kozielski F.* Structural links to kinesin directionality and movement // Nat. Struct. Biol. — 2000. — **7**. — P. 456–460.
68. *Endow S.A., Higuchi H.* A Mutant of the motor protein kinesin that moves in both directions on microtubules // Nature. — 2000. — **406**. — P. 913–916.
69. *Block S.M., Goldstein L.S., Schnapp B.J.* Bead movement by single kinesin molecules studied with optical tweezers // Nature. — 1990. — **348**. — P. 348–352.
70. *Crevel I.M., Lockhart A., Cross R.A.* Kinetic evidence for low chemical processivity in ncd and Eg5 // J. Mol. Biol. — 1997. — **273**. — P. 160–170.
71. *Hancock W.O., Howard J.* Processivity of the motor protein kinesin requires two heads // J. Cell Biol. — 1998. — **140**. — P. 1395–1405.
72. *Ray S., Meyhufer E., Milligan R.A., Howard J.* Kinesin follows the microtubule's protofilament axis // J. Cell Biol. — 1993. — **121**. — P. 1083–1093.
73. *Howard J.* The movement of kinesin along microtubules // Annu. Rev. Physiol. — 1996. — **58**. — P. 703–729.
74. *Kojima H., Muto E., Higuchi H., Yanagida T.* Mechanics of single kinesin molecules measured by optical trapping nanometry // Biophys J. — 1997. — **73**. — P. 2012–2022.
75. *Nishiyama M., Muto E., Inoue Y., Yanagida T., Higuchi H.* Substeps within the 8-nm step of the ATPase cycle of single kinesin molecules // Nat. Cell Biol. — 2001. — **3**. — P. 425–428.
76. *Schief W.R., Howard J.* Conformational changes during kinesin motility // Curr. Opin. Cell Biol. — 2001. — **13**. — P. 19–28.
77. *Schnitzer M.J., Block S.M.* Kinesin hydrolyses one ATP per 8-nm step // Nature. — 1997. — **388**. — P. 386–390.
78. *Schnapp B.J., Crise B., Sheetz M.P., Reese T.S., Khan S.* Delayed start-up of kinesin-driven microtubule gliding following inhibition by adenosine 5'-[beta,gamma-imido] triphosphate // Proc. Nat. Acad. Sci. USA. — 1990. — **87**. — P. 10053–10057.
79. *Steinberg G., Schliwa M.* Characterization of the biophysical and motility properties of kinesin from the fungus Neurospora crassa // J. Biol. Chem. — 1996. — **271**. — P. 7516–7521.
80. *Massow A. von., Mandelkow E.M., Mandelkow E.* Interaction between kinesin, microtubules, and microtubule-associated protein 2 // Cell Motil. Cytoskel. — 1989. — **14**. — P. 562–571.
81. *Böhm K.J., Stracke R., Unger E.* Speeding up kinesin-driven microtubule gliding in vitro by variation of cofactor composition and physicochemical parameters // Cell Biol. Int. — 2000. — **24**. — P. 335–341.
82. *Böhm K.J., Stracke R., Baum M., Zieren M., Unger E.* Effect of temperature on kinesin-driven microtubule gliding and kinesin ATPase activity // FEBS Lett. — 2000. — **466**. — P. 59–62.
83. *Kawaguchi K., Ishiwata S.* Thermal activation of single kinesin molecules with temperature pulse microscopy // Cell Motil. Cytoskel. — 2001. — **49**. — P. 41–47.
84. *Hunt A.J., Gittes F., Howard J.* The force exerted by a single kinesin molecule against a viscous load // Biophys. J. — 1994. — **67**. — P. 766–781.
85. *Hall K.W., Cole D.G., Yeh Y., Scholey J.M., Baskin R.J.* Force generation in kinesin measured in a centrifuge

microscope-based motility assay // Biophys. J. — 1995. — **68**. — P. S71.
86. *Svoboda K., Block S.M.* Force and velocity measured for single kinesin molecules // Cell. — 1994. — **77**. — P. 773–784.
87. *Crevel I., Carter N., Schliwa M., Cross R.* Coupled chemical and mechanical reaction steps in a processive Neurospora kinesin // EMBO J. — 1999. — **18**. — P. 5863–5872.
88. *Kawaguchi K., Ishiwata S.* Temperature dependence of force, velocity, and processivity of single kinesin molecules // Biochem. Biophys. Res. Communs. — 2000. — **272**. — P. 895–899.
89. *Turner D.C., Chang C., Fang K., Brandow S.L., Murphy D.B.* Selective adhesion of functional microtubules to patterned silane surfaces // Biophys. J. — 1995. — **69**. — P. 2782–2789.
90. *Dennis J.R., Howard J., Vogel V.* Molecular shuttles: directed motion of microtubules along nanoscale kinesin tracks // Nanotechnol. — 1999. — **10**. — P. 232–236.
91. *Limberis L., Stewart R.J.* Toward kinesin-powered microdevices // Nanotechnol. — 2000. — **11**. — P. 47–51.
92. *Stracke R., Böhm K.J., Burgold J., Schacht H.J., Unger E.* Physical and technical parameters determining the functioning of kinesin-based nanoactuators // Nanotechnol. — 2000. — **11**. — P. 52–56.
93. *Böhm K.J., Stracke R., Mühlig P., Unger E.* Motorprotein-driven unidirectional transport of micrometer-sized cargoes across isopolar microtubule arrays // Nanotechnol. — 2001. — **12**. — P. 238–244.
94. *Hiratsuka Y., Tada T., Oiwa K., Kanayama T., Uyeda T.Q.P.* Controlling the direction of kinesin-driven microtubule movements along microlithographic tracks // Biophys. J. — 2001. — **81**. — P. 1555–1561.
95. *Turner D., Chang C.Y., Fang K., Cuomo P., Murphy D.* Kinesin movement on glutaraldehyde-fixed microtubules // Anal. Biochem. — 1996. — **242**. — P. 20–25.
96. *Howard J., Hudspeth A.J., Vale R.D.* Movement of microtubules by single kinesin molecules // Nature. — 1989. — **342**. — P. 154–158.
97. *Coy D.L., Wagenbach M., Howard J.* Kinesin takes one 8-nm step for each ATP that it hydrolyzes // J. Biol. Chem. — 1999. — **274**. — P. 3667–3671.
98. *Hess H., Clemmens J., Qin D., Howard J., Vogel V.* Light-controlled molecular shuttles made from motor proteins carrying cargo on engineered surfaces // Nano Lett. — 2001. — **1**. — P. 235–239.
99. *Pollock N., Hostos E.L. de, Turck C.W., Vale R.D.* Reconstitution of membrane transport powered by a novel dimeric kinesin motor of the Unc104/KIF1A family purified from Dictyostelium // J. Cell Biol. — 1999. — **147**. — P. 493–505.
100. *Böhm K.J., Stracke R., Vater W., Unger E.* Inhibition of kinesin-driven microtubule motility by polyhydroxy compounds // Micro- and Nanostructures of Biological Systems /Eds H.-J. Hein, G. Bischoff. — Shaker Verlag Aachen, 2001. — P. 153–165.

Received 10.01.03