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Review

Directional motility of kinesin motor proteins

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Abstract

Kinesin motor proteins are molecules capable of moving along microtubules. They share homology in the so-called core motor domain which acts as a microtubule-dependent ATPase. The surprising finding that different members of the superfamily move in opposite directions along microtubules despite their close similarity has stimulated intensive research on the determinants of motor directionality. This article reviews recent biophysical, biochemical, structural and mutagenic studies that contributed to the elucidation of the mechanisms that cause directional motion of kinesin motor proteins.

Keywords: Molecular motor; Kinesin; Non-claret disjunctional protein; Directionality; Processivity; Microtubule

1. Introduction

There are three filament-dependent motor protein superfamilies known so far: myosins, dyneins and kinesins. They move along actin (myosin family) or microtubules (dynein and kinesin families), respectively [1]. No motor proteins for intermediate filaments are known; apparently, polarity of the filament is a prerequisite for proteins to move in a directed manner along a substrate, and intermediate filaments are not known to be polar structures. Conventional kinesin had been shown early on to move towards the plus-end of microtubules and the discovery that the kinesin-related non-claret disjunction (ncd) motor protein moves in the opposite direction came as a surprise [2,3]. Until today, the basis for the directionality of kinesin-related motor proteins is under investigation. From a biophysical perspective, it would be interesting to know whether directed motion arises from biased diffusion (entirely based on structural asymmetry, or using some sort of ratchet), or by active propulsion of the molecule. From a biochemist's viewpoint, two basic questions arise: (i) how is directed motion linked to the kinetic cycle of the motor protein, and (ii) what are the structural requirements for proper function? The structural basis can be addressed by means of molecular biology or high-resolution imaging methods such as electron microscopy (EM) or X-ray crystallography. This review summarizes the main concepts and describes experimental approaches to probe the question of kinesin directionality.

1.1. Kinesin-driven motility

Initially, kinesin was discovered in neuronal tissue and was found to move microtubules in a microscopic in vitro gliding assay in an ATP-dependent fashion. Native squid kinesin as well as other related animal kinesins are tetrameric proteins consisting of

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two identical heavy and two identical light chains. The motor activity was shown to reside in the heavy chains.

Characterization of heavy chain constructs truncated at the C-terminal end revealed domains essential for motility: the globular N-terminal ~320 amino acids ('head', due to its EM appearance), possesses microtubule-activated ATPase activity ('catalytic core', or 'core motor domain') but seems to be insufficient for motility. It is linked to a coiled-coil region ('neck', by analogy) which causes the molecule to dimerize via a short linker sequence. Motor constructs that contain the neck exhibit processive motility similar to wild-type. Further C-terminal, a 'hinge' of presumably flexible, extended conformation connects to the 'stalk' and 'tail' domains which are thought to be involved in cargo binding but apparently are not actively involved in generation of motility. The hinge region, however, may be an element conferring optimal performance [4].

The type of kinesin described so far is nowadays classified as conventional kinesin and turned out to be the founding member of a large superfamily defined by homology in the catalytic core. In addition, several other subfamilies have been defined based on their domain arrangement, sequence similarity and oligomerization state. Outside of the catalytically active core motor domain, kinesin and kinesin-related proteins diverge, reflecting their distinct biological roles which cover a wide range of activities, including intracellular organelle and vesicle transport, as well as in chromosome movement.

With respect to their motile properties, conventional kinesins from *Drosophila*, rat and human are investigated most extensively and have been shown to move processively for many μm to microtubule plus-ends. None of the conventional kinesins moves in the opposite direction which is not extraordinarily surprising because of the close similarity over the entire amino acid sequence. All known minus-end-directed kinesins are members of the C-terminal subfamily and share common sequence motifs. Conversely, all C-terminal kinesin motors whose motile properties have been determined move to microtubule minus-ends. The *Drosophila* ncd protein is the best studied representative of this family and most studies addressing the problem of directionality have used ncd.

2. Biophysical properties of kinesin motor proteins

The question how directed motion is achieved is intimately connected with the general problem of how force is generated. Naively, one may think that kinesin works similar to myosin. The minimal functional myosin molecule, the S1 fragment, comprises the core motor domain with ATP and filament binding sites, and a long C-terminal α-helix which is stabilized by associated light chains ('light chain binding domain'). In the course of its reaction cycle, the molecule attaches to F-actin in an ADP/phosphate-containing state. While assuming a strongly bound state with a defined orientation on the filament, it loses phosphate and nucleotide. According to cryo-EM image reconstructions of smooth muscle myosin and brush border myosin, force generation is based on a swinging motion of the light chain binding domain upon ADP release [5–7]. Apparently, myosins use a lever mechanism to amplify small conformational changes in the ATPase domain by virtue of an additional 'converter domain' in the myosin head [8]. Here, directionality is imposed by the defined orientation of the S1 molecule in its strong binding state along the actin filament and the geometry of the pivot axis.

2.1. Single molecule processivity

For kinesin, the picture appears not to be so simple. Although kinesin's neck coiled-coil at first glance resembles the myosin light chain binding domain, its deletion does not abolish processive motility and its duplication does not accelerate the gliding velocity [9]. Moreover, in addition to explaining the generation of a forward bias, a model describing kinesin motility needs to address the question of how processivity is achieved. For conventional kinesins, it has been shown that motility is generated by multiple consecutive 8 nm steps. In between steps, the kinesin dimer remains firmly attached to the filament with one of its 'heads'. In addition to a tentative 'lever swing', a mechanism ensuring continuous binding of at least one of the two heads needs to be included in the model.

The characteristics of processive motility of single molecules has first been observed in gliding assays on glass surfaces and has later been characterized further

using laser trap microscopy [10,11]. In these experiments, latex beads sparsely coated with kinesin were captured in a laser beam and the position of the bead was followed over time. Above the thermal background, discrete plateaus could be detected that lay 8 nm apart. Several consecutive steps were observed until the bead reached an off-center position where the force exerted by the laser trap equaled the force generated by a single kinesin molecule. The kinesin maximum force thus determined is around 5–6 pN (reviewed by Howard [12]). The laser trapping experiments have been interpreted to reflect intermediates in the reaction cycle with a strongly bound rate-limiting state being visible as a plateau. Myosin, in contrast, falls off the filament after the power stroke.

Structurally, this implies an intermediate state of the kinesin dimer that bridges 8 nm along the microtubule. Therefore, a 'hand-over-hand' model of kinesin motion has been proposed [10]: while one kinesin head is being bound to the microtubule, the other one 'reaches' towards a forward binding site.

2.2. Ratchet

How does directionality relate to this model? The laser trapping experiments show an additional interesting feature: backward steps seem to be prevented. Apparently, the (dimeric) molecule acts as a ratchet and prevents reversion of the stepping process. Coppin et al. [13] studied the behavior of conventional kinesin under external backward forces and found at most one single backward step. They attribute this phenomenon to phase shifts in the reaction cycles between the anterior and posterior heads: if the rear head is strongly bound while exerting an external backward force, the front head has a high probability to detach instead of transiting to a strongly bound state at the forward site. Single backward steps have not been confirmed by other groups but it seems undisputed that kinesin's stepping mechanism is intrinsically irreversible.

2.3. Energetic considerations

These biophysical data imply that at all times during processive motion, at least one head remains strongly bound to its microtubule substrate. The energy required to overcome the binding force and the decrease in entropy (directional rebinding obviously is a highly ordering process) may be derived from thermal (Brownian motion) or chemical energy (i.e. ATP hydrolysis). Many (bio)physicists tend to favor the first possibility whereas biochemists seem to prefer the latter one. If the energy for stepping was derived from thermal fluctuations, according to the first law of thermodynamics, the heat used to accommodate the step would have to be returned to the environment. Here, the enthalpy from ATP hydrolysis would come into play, though in a very abstract manner. Biochemists usually imagine chemical energy being stored in an (otherwise unfavorable) conformation which relaxes while doing work. Though thermodynamically equivalent, the latter way of modeling may offer a structural basis for understanding mutant phenotypes.

2.4. Possible models for directional stepping

Knowing that kinesin motility is based on discrete steps along the microtubule substrate, the following models can be considered to understand directionality (Fig. 1):

- First, a model involving a lever similar to myosin may explain the observation (discussed by [14]). As Fig. 1a indicates, the bound head may push the other head to a forward binding site. Here, the lever swing would not necessarily have to occur perpendicular to the filament axis; the lever may as well swing laterally and propel the other head by a torsional movement (Fig. 1a).
- Alternatively, motility may depend on a defined linkage between bound and free head that positions the unbound head closer to the 'forward' binding site. Upon induction of a stepping event, this head would have a high probability of directed rebinding (Fig. 1b).
- It has been suggested that tubulin may play an active role and allosterically affect neighboring heterodimers upon kinesin binding [15]. This way, one direction might be favored for binding (Fig. 1c).
- Finally, a specific domain connecting the two core motor domains may be associated with the posterior head before the 'power stroke' and change its coordination to the anterior head afterwards. This domain might be identical to what has been

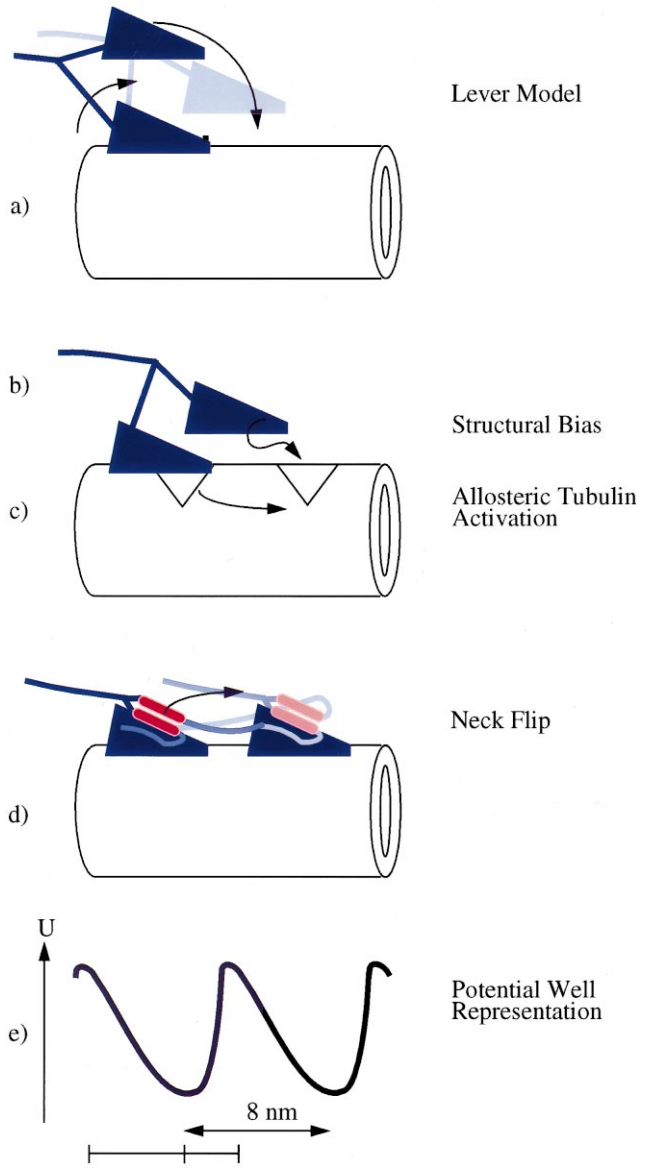

Fig. 1. Possible models for directional kinesin motility. The first model (a) assumes a rigid connection between the two kinesin heads which is propelled forward by the action of the bound head. In (b), the head-head linkage is flexible enough to allow tethered diffusion but stiff enough to position the unbound head closer to a forward binding site. Model (c) implies activation of the forward tubulin subunit upon kinesin binding which may favor the forward site for rebinding. The last model (d) emphasizes that stepping and head binding events may be uncoupled and rearrangement of the neck region may also cause a forward net movement. Energetically, all four models can be described by a potential well representation (e).
←

termed neck-linker, or it might be the neck itself and potential additional elements. This model, called 'linker flip' model in Fig. 1d, discerns the force generation and the microtubule unbinding/rebinding events because tight binding of the connector element to the forward head would correspond to step without requiring the posterior head to detach. It could explain kinetic differences [16] between the two heads by the associated or dissociated state of the head connector region and the core motor domain. Structurally, it may be in agreement with the observations of Hoenger, Mandelkow et al. [17] and the mutagenesis study by Romberg [9].

All these models rely on elements of tethered diffusion and conformational change to different degrees. Diffusion is a largely passive, physical principle whereas conformational change implies an active response of the protein. The latter component could easily introduce a directional bias whereas tethering might explain the processivity of kinesin. The extend of active and passive elements in molecular motility is certainly a crucial issue for future investigations on directionality.

Apart from these schematic visualizations, the directional bias of kinesin-based motility can be described using potential curves (Fig. 1e). The strongly bound head is kept at the energetic minimum ('potential well') whereas the second head may diffuse in the potential field. Here, it is more likely to cross the closer 'rim' which is located in the forward direction of motility. Once it has entered the next, unoccupied potential well, it 'falls down' to the new energetic minimum.

Essentially, either representation expresses similar ideas. The potential well description may be more suitable for quantitative measurements using laser trap setups, whereas a structural sketch may help to understand domain functionality.

It should be emphasized that these considerations only apply to dimeric processive motors. Non-processive motors not necessarily need to bridge two successive binding sites and their multiple motor motility may as well be derived from small directional biases of a single molecule. In fact, for the best characterized minus-end-directed kinesin-related motor, ncd, neither processivity nor 8 nm steps have been demonstrated and, therefore, a different explanation may apply.

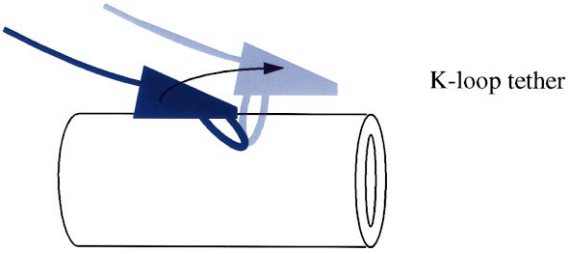

Fig. 2. Motility of monomeric Kif1A kinesin based on the hypothetical K-loop tether (see text for details).

2.5. Monomeric kinesin motility

Recently, motility of a monomeric kinesin-related motor protein has been reported [18]. The Kif1A subfamily of kinesin motors apparently does not dimerize and it has been unclear whether they are capable of processive motility. Okada and Hirokawa used a genetically engineered version of this motor containing the catalytic core of Kif1A fused to the *Drosophila* kinesin neck-linker (which is insufficient for dimerization), and observed single molecule run lengths of 840 nm, corresponding to approximately 100 8 nm steps on average. Here, the directionality displayed an interesting behavior: whereas conventional kinesin showed only minor backward displacements probably due to instrumental inaccuracy, the Kif1A derivative exhibited a net plus-end-directed motility with temporal phases of reverse motility. The traces fit a model of stochastic biased diffusion. The authors suggest that processivity of this monomeric kinesin may be explained by a specialized, lysine-rich loop (K-loop) in the motor core that may tether the molecule at the microtubule during the stepping process (Fig. 2). The loose directional bias may be an intrinsic property of this particular kinesin subgroup, or may be due to the artificial neck sequence present in the construct.

3. Kinetics

Several kinetic studies have demonstrated that kinesin's nucleotide and microtubule binding states are kinetically coordinated [19–21].

For the understanding of directionality determination, it is important to know that the two heads of a conventional kinesin dimer differ kinetically. Artificially monomeric kinesin constructs rapidly loose their one ADP molecule upon microtubule binding. In solution, ADP is being exchanged extremely slowly. Accordingly, it has been concluded that microtubules trigger the release of bound nucleotide. In dimeric constructs, only one ADP out of two is being released which suggests that only one head can bind to microtubules under these conditions. The other one is protected from exchanging ADP by an unknown mechanism of steric or kinetic inhibition. After the addition of ATP, excess ADP or AMPPNP, the kinetic arrest is removed and the second ADP is being exchanged at the second kinesin head.

3.1. Kinetics of ncd vs. kinesin

There are models that attribute the different directionalities of kinesin and ncd to kinetic differences [22]. Kinesin assumes a tightly microtubule-bound conformation in the nucleotide-free state. In a dimer, the unbound (or loosely bound) head contains ADP and displays weak microtubule affinity. Assuming that for ncd the situation was vice versa, the ADP state was tightly bound and the nucleotide-free state loosely bound, then reversal of directionality might be achieved with an otherwise similar head-head coordination (Fig. 3).

However, this seems not to be the case. On the contrary, ncd seems to have very similar (though overall slower) kinetic properties, with the microtubule-activated ADP release being the rate-limiting step and the nucleotide-free microtubule ncd inter-

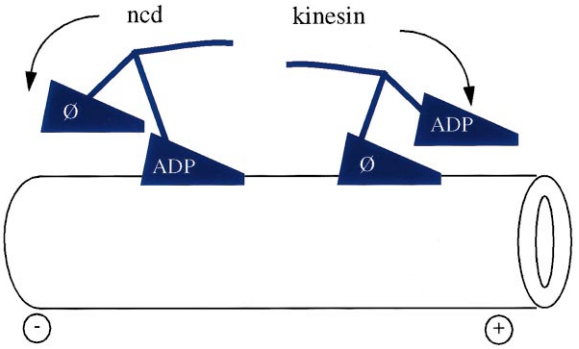

Fig. 3. Kinetic model of reversal of directionality. Two molecular motors could have opposite directionality if their kinetic states were swapped. For kinesin and ncd, however, this seems not to be the case [22]. See text for details.

mediate being a very stable complex [23,24]. Because of the instability of the ncd molecule, the stoichiometry of the ADP release upon microtubule binding is unclear. In summary, minus-end-directed directionality of ncd appears not to be a consequence of kinetic differences [22,25–27].

3.2. Binding studies

Directionality may originate in different orientations of kinesin and ncd along the microtubule. Competition experiments, however, have shown that both molecules use at least overlapping binding sites [28–30]. Proteolysis protection assays have shown that kinesin and ncd use conserved residues for microtubule binding [31]. Together with EM studies (see below), it seems unlikely that the opposite directionality of kinesin and ncd is due to an altered binding orientation.

4. EM and crystal structure

In the case of myosin, cryo-EM studies strongly support the lever arm hypothesis. Therefore, several analogous studies have been performed on kinesins. In these investigations, constructs of different lengths of conventional kinesin (from different organisms) and ncd have been used: proteins comprising amino acids 1–350 (approximately) of the motor domain are monomeric, longer ones possess the neck domain which causes dimerization. Whereas for the attachment of monomers a wide agreement has been achieved, the interpretation of dimeric data is still controversial [32,33]. We therefore treat both cases separately.

4.1. Reconstructions of monomeric kinesins

The first cryo-EM reconstruction studies have been performed on monomeric kinesin and ncd constructs. In either case, similar patterns became visible: the core motor domains attach at slightly tilted angles somewhat sidewise to the crest of a protofilament. The motor heads are somewhat reminiscent of droplets with their pointed ends oriented to the microtubule plus-end. The contact surface seems to extend over both tubulin subunits although one tubulin monomer, most likely the β-subunit, is covered more extensively [15,34,35]. These and later data indicate that directionality of either motor is not due to different attachment along the filament [36].

Hirose et al. [34,37] varied the nucleotide conditions and found small structural changes in a small extension formed by kinesin on the opposite side of its microtubule binding interface. According to their model, directionality might originate in this tiny conformational change of a kinesin monomer and might be amplified by additional structures present only in dimeric kinesins to yield 8 nm steps. In a recent study using dimeric motors, the authors supported their view of nucleotide-dependent structural changes in the bound head [33]. It remains unclear, however, whether the observations reflect a significant rearrangement.

Fitting the monomeric crystal structure of the ncd core motor domain (see below) into the corresponding EM reconstruction images indicated that the tip of the ncd 'droplet' corresponds to loop L10 and that the microtubule binding regions as identified by mutagenesis and protease protection assays actually can be modeled on top of the microtubule surface [38]. The tip of the ncd monomer points towards the microtubule plus-end. Alternative models [39] that predict a different microtubule interface do not agree with the biochemical data and predict poorly conserved residues to be involved in binding.

4.2. Dimeric kinesin-microtubule decoration

For dimeric kinesin motors, divergent positions of the second, unbound head of kinesin and ncd, respectively, were observed [40–42]. Although in the case of kinesin the position of the second head remains controversial, some authors conclude that the second head is oriented towards the microtubule plus-end. For ncd, the free head is well ordered and points sidewise. In either case, relief of the kinetic arrest, caused by ATP binding of the nucleotide-free, bound head, may lead to a biased rebinding just by the quicker diffusion towards the closer binding site. Here, the formerly unbound head could transit to a nucleotide-free, strong microtubule-bound state while the other head hydrolyzes its 'new' ATP and transits into a weak binding state.

However, these interpretations of the EM data are

not undisputed because for kinesin, the additional mass visible in cryo-EM images was significantly smaller than expected and might be attributed to the additional mass introduced by only the neck [17]. This view was supported by kinesin-microtubule binding experiments [43] which suggest that dimeric kinesin binds to microtubules in a ~1:2 stoichiometry (one kinesin monomer per tubulin dimer) and displays a 16 nm spacing in X-ray scattering experiments. Hence, the authors conclude that under their conditions, the kinesin dimer may cover two adjacent tubulin heterodimers. Directionality might be based on the distinction of forward and rear heads due to their different kinetic states.

The 16 nm periodicity, however, has not yet been observed by others, and binding stoichiometries of 1:1 (one kinesin dimer per one tubulin heterodimer) have been measured [28,44].

Hence, it remains unclear whether EM reconstructions allow to draw conclusions about directional biases. Some authors favor a tethered diffusion model with a structural bias imposed by the bound head on the unbound one. Whereas a ~180° rotation around the longitudinal axis of the head during the reaction cycle (as implied by Kozielski et al.) seems unlikely, there is experimental support for a model resembling the 'neck-flip' model. The second, kinetically inactive head might actually be weakly bound to a forward microtubule binding site and stepping may occur by association of the neck (or other elements) to the forward head. This change of neck coordination may be induced by ATP binding of the first head.

4.3. X-ray crystal structures

The solution of X-ray crystal structures has provided additional information on how a kinesin motor might move. Today, three-dimensional crystal structures of monomeric and dimeric conventional kinesin and ncd are available [45–48] and need to be incorporated into models explaining motility. Also, the monomeric structure of the minus-motor kar3 is known [49]. Basically, the core motor domain which is responsible for ATP hydrolysis and nucleotide-dependent microtubule binding [44,50,51] is very similar for plus- and minus-motors. It has been proposed that ATP hydrolysis in kinesins, as in myosins and G-proteins, also involves loops (termed switches I and II in the G-protein field) that position the catalytic water molecule depending on the presence or absence of an allosteric protein partner. For G-proteins, this process seems to involve both switch regions as well as the so-called switch II helix because their positions differ in the crystal structures of the diphosphate and (presumable) triphosphate states. Interestingly, the switch II helix ends close to the predicted microtubule binding site and may couple conformational changes in the microtubule and nucleotide binding sites. In a simulation study, it has been predicted that when the microtubule binding site is immobilized at the microtubule, the longitudinal axis of the kinesin core motor domain may tilt by several degrees [52]. Because of the structural and kinetic similarity of the kinesin-like core motor domains, it is unlikely that the plus- and minus-end-directed motors differ significantly.

In contrast, the regions adjacent to the motor cores differ significantly in conventional kinesin and ncd. In both cases, crystals were obtained of constructs comprising some 40 amino acids of the respective neck region in addition to the catalytic motor domain; in both cases, these residues form coiled-coils and are responsible for dimerization. However, the primary sequences of these 'necks' are not homologous and are fused to the opposite terminus of the respective core motor. In kinesin, a stretch of approximately 12 amino acid residues termed 'neck-linker' hooks up the core motor domain and the neck coiled-coil. It interacts with residues in the core motor domain. Although ncd does not possess a similar neck-linker, the homologous residues in the motor core apparently do have a function: they interact with the neck coiled-coil directly. As a consequence of their different necks, the dimeric structures also differ significantly: whereas in conventional rat kinesin the two core motor domains point into directions that span approximately 120° with the neck pointing in a third, different direction, the ncd core domains fold back onto the neck coiled-coil. As a whole, the conventional kinesin resembles a Y whereas the ncd dimer looks like a twig with two dry leaves hanging from its end.

The dimeric crystal structures do not easily fit into the EM reconstructions. In the EM, the microtubule-attached heads of kinesin as well as ncd display

shapes that can be superimposed with the corresponding crystal structures. The tip of the head 'droplet' corresponds to loop 10, the blunt end is located close to loop 14/helix α4. Obviously, the microtubule interface should be located radially to this longitudinal axis. Currently, there are three different interpretations of EM reconstructions that place the microtubule binding interface at different angles. Whereas Hoenger's and Mandelkow's, as well as Hirose's, Amos' and Cross' models agree with biochemical and mutagenic data, Wade et al. predict protease-sensitive residues to be solvent-exposed that cause, when mutated, microtubule interaction defects [17,33,38,39,50,51].

The rotational position of the bound head also affects the location of the second head and the neck coiled-coil. Hoenger's and Mandelkow's reconstructions lead to an interference of the kinesin neck coiled-coil with the microtubule surface, the other authors avoid these steric problems by rotation of the bound head. Due to the lack of reliable fitting algorithms for crystal and EM structures, and due to the lack of knowledge of the structural rearrangements of kinesin upon microtubule binding, it is hard to decide which model fits the observations most closely. To discriminate between the different possibilities, knowledge about the additional electron density present in dimeric constructs would be helpful. As mentioned above, the volume of this density is smaller than the second head which could be attributed to a disordered state, or to the additional mass introduced by the dimerization domain (neck). Here, too, no agreement has been achieved.

In the case of ncd, interpretation is easier because the unbound head is well ordered and exhibits the expected size. To match the dimeric crystal structure with EM structures, a twist of the free head relative to the bound head has to be postulated upon microtubule binding. Possibly, ADP release may induce this torsional movement.

In summary, high-resolution structural data may be in agreement with either a 'structural bias' or a 'linker flip' model of motility. A lever arm comparable to myosin has not yet been identified. The lack of sufficient densities in EM reconstructions in the case of the second kinesin head may be due to statistic variations and hint at a tethered diffusion event. Small conformational changes upon microtubule binding and/or ADP release may induce an (amplified?) structural bias that favors 'forward' rebinding. Alternatively, one kinesin dimer may bridge two microtubule binding sites which, however, may represent different kinetic states. The kinesin stalk may be dragged by flipping the dimerization domain (most likely the neck) from 'rear' to 'front' head.

5. Mutational studies

In a seminal study, Stewart et al. [53] mapped the domains responsible for directed motility. Using conventional *Drosophila* kinesin and ncd, they constructed a series of fusion proteins between motor and spectrin (kinesin) and glutathione transferase (kinesin and ncd), respectively. The chimeras contained the homologous part of the molecules, now generally termed catalytic motor domain, and flanking regions of variable length. That way, the authors were able to determine the minimal sequence requirement for directional motility. According to current terminology, they found that core motor domain as well as neck-linker (for kinesin) and neck (for ncd) were necessary to promote correct gliding behavior. The velocities were lower than for wild-type protein, but additional sequence accelerated the molecules close to wild-type speeds (Fig. 4). Interestingly, the conventional kinesin motor produced plus-end-directed motility even when fused to the C-terminus of the spectrin polypeptide chain, indicating that the topology of the molecule per se is non-essential for direction of motility.

Constructs lacking kinesin's neck-linker or ncd neck were not investigated by Stewart and coworkers. Therefore, later studies used chimeric kinesin-ncd constructs fused at different points (Fig. 4, [54–57]). Case et al. [54] as well as Henningsen and Schliwa [55] managed to clone chimeric motors based on the ncd core motor domain with plus-end-directed motility. In both cases, the neck of conventional kinesin and most of the neck-linker were present in the chimera whereas the N-termini differed: Case et al. fused their construct to the human kinesin N-terminus, Henningsen and Schliwa used an N-terminally truncated ncd version. The thorough study by Endow and Waligora [57], however, showed that the kinesin neck is not an indispensable prerequisite for

plus-end motility: a construct comprising the *Drosophila* conventional kinesin motor core 'sandwiched' by ncd sequences and lacking most of the kinesin neck and neck-linker still moved to the plus-end of microtubules. In contrast, when the same construct with the N-terminal fusion point shifted by only two amino acids was used, the direction of motility was reversed. Comparison with the dimeric ncd crystal structure [48] reveals that these two crucial glycine-asparagine residues from ncd terminate the ncd neck helix and may confer flexibility between neck and motor core. Sablin et al. [48] also report a mutant with a disrupted ncd neck helix and without domains derived from conventional kinesin that moves in the microtubule plus-end direction. Therefore, it seems that a proper ncd neck helix and motor core junction are necessary for minus-end-directed motility. According to the crystal structure, the ncd neck interacts with residues conserved in the entire superfamily in the motor core, and therefore the ncd neck and joint may even be sufficient to promote minus-end-directed motility, although direct proof is lacking.

Conversely, since there are chimeric, plus-end-directed motor constructs that lack kinesin's neck and neck-linker, the contribution of these domains for

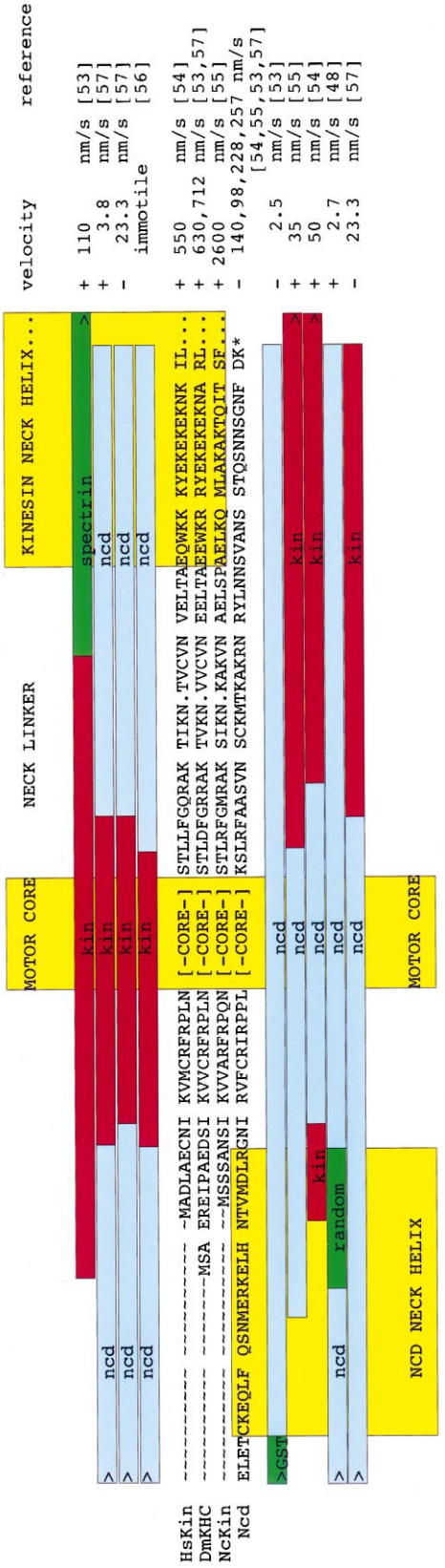

Fig. 4. Schematic representation of chimeric kinesin and ncd constructs used for determination of directionality. Relevant domains of conventional kinesins from human (HsKin), *Drosophila* (DmKHC), *Neurospora crassa* (NcKin) and the C-terminal kinesin motor ncd (ncd) are aligned and functional domains (neck helices of kinesin and ncd, as well as the core motor domains) are emphasized by textured boxes. Above the alignment, chimeric kinesins with a core motor domain from a conventional kinesin are shown, below the alignment, constructs possessing the ncd motor core are grouped. Light gray bars symbolize ncd sequences, dark ones conventional kinesin, intermediate gray signifies foreign sequences. At the right border, velocities with references are given (positive values indicate microtubule plus-end motility). It is obvious that the motor core does not determine the directionality of the molecular motor but flanking sequences. For minus-end-directed motility, an intact ncd neck helix appears to be essential whereas for plus-end-directed motility, no simple requirement can be deduced. It is also obvious that none of the chimeric motors reaches wild-type gliding velocities. Note that Case et al. [54] used human kinesin, Henningsen and Schliwa *Neurospora* kinesin [55], Stewart et al. [53] and Endow and Waligora [57] *Drosophila* kinesin as a source of conventional kinesin sequences. For further discussion, see text.

directionality is more difficult to assign. All motile fusion proteins that have been investigated still contain either part of flanking kinesin sequences, or the ncd C-terminal 'appendix'. Ncd possesses about 40 C-terminal, non-conserved amino acids that display slight similarity to the kinesin neck-linker (Fig. 4). It is therefore unclear whether this domain is able to substitute the original domain, or whether the kinesin core motor domains possess a 'default' plus-end directionality. The kinetic behavior of conventional kinesin with and without neck-linker differs significantly which argues for a functional role of this domain [16]. Also, in the crystal structure, there are contacts visible between the neck-linker and core motor domain suggesting a specific mutual influence. Further studies using novel neck variants may help to solve the question.

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