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Back on track – On the role of the microtubule for kinesin motility and cellular function

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Abstract

The evolution of cytoskeletal filaments (actin- and intermediate-filaments, and the microtubules) and their associated motor- and non-motor-proteins has enabled the eukaryotic cell to achieve complex organizational and structural tasks. This ability to control cellular transport processes and structures allowed for the development of such complex cellular organelles like cilia or flagella in single-cell organisms and made possible the development and differentiation of multi-cellular organisms with highly specialized, polarized cells. Also, the faithful segregation of large amounts of genetic information during cell division relies crucially on the reorganization and control of the cytoskeleton, making the cytoskeleton a key prerequisite for the development of highly complex genomes. Therefore, it is not surprising that the eukaryotic cell continuously invests considerable resources in the establishment, maintenance, modification and rearrangement of the cytoskeletal filaments and the regulation of its interaction with accessory proteins. Here we review the literature on the interaction between microtubules and motor-proteins of the kinesin-family. Our particular interest is the role of the microtubule in the regulation of kinesin motility and cellular function. After an introduction of the kinesin–microtubule interaction we focus on two interrelated aspects: (1) the active allosteric participation of the microtubule during the interaction with kinesins in general and (2) the possible regulatory role of post-translational modifications of the microtubule in the kinesin–microtubule interaction.

Kinesin–microtubule interaction

Kinesins are a large and diverse family of microtubule based motors (Vale, 2003; Lawrence *et al*., 2004). They all share a high degree of sequence homology in the motor domain with the founding member, conventional kinesin (Kinesin-1) (Brady, 1985; Vale *et al*., 1985). Conventional kinesins are (+)-end directed motors that are hetero-tetramers of two heavy and two light chains (Kuznetsov *et al*., 1988). The N-terminal motor domains of Kinesin-I contain both the ATPase and MT-binding activity (Kuznetsov *et al*., 1989; Scholey *et al*., 1989). They are flexibly linked to the α-helical neck via the so-called neck-linker, which is believed to confer important conformational changes during the generation of movement (Rice *et al*., 1999). Coiled-coil interactions between the neck domains of two heavy chains lead to dimerization of Kinesin-1 (de Cuevas *et al*., 1992). An extended coiled-coil of about 60 nm forms the stalk, which is interrupted by flexible hinges and kinks (Kirchner *et al*., 1999). A small globular, C-terminal tail is responsible for light chain (KLC) binding (Johnson *et al*., 1990; Verhey *et al*., 1998). Tail and KLC are important for both the binding of various cargo adapters and regulation of the unbound motor through inhibitory back-folding (Goldstein, 1993; Verhey *et al*., 1998; Coy *et al*., 1999; Seiler *et al*., 2000; Goldstein, 2001).

Kinesin-I has the remarkable ability to coordinate the ATPase activity of its two heads such that a single molecule is capable of moving processively along the microtubule for micrometer distances (Howard *et al*., 1989; Hackney, 1994; Vale *et al*., 1996; Gilbert *et al*., 1998; Lakamper *et al*., 2003). By alternately advancing the motor domains along the microtubule in a hand-over-hand fashion Kinesin-1 can take hundreds of 8 nm steps and produce stalling forces of up to 5–7 pN (Svoboda *et al*., 1993; Meyhofer and Howard, 1995; Asbury, 2003; Yildiz *et al*., 2004; Carter and Cross, 2005). Processivity is enabled by strictly asynchronous, ATP-driven cycling between weak and strong binding states of each of the two heads. While ATP-bound and nucleotide-free motor-domains are strongly bound to the microtubule, ADP-bound heads are weakly bound. A working hypothesis for this mechano-chemical cycle is summarized in Figure 1. In solution, both heads of dimeric kinesin are in the

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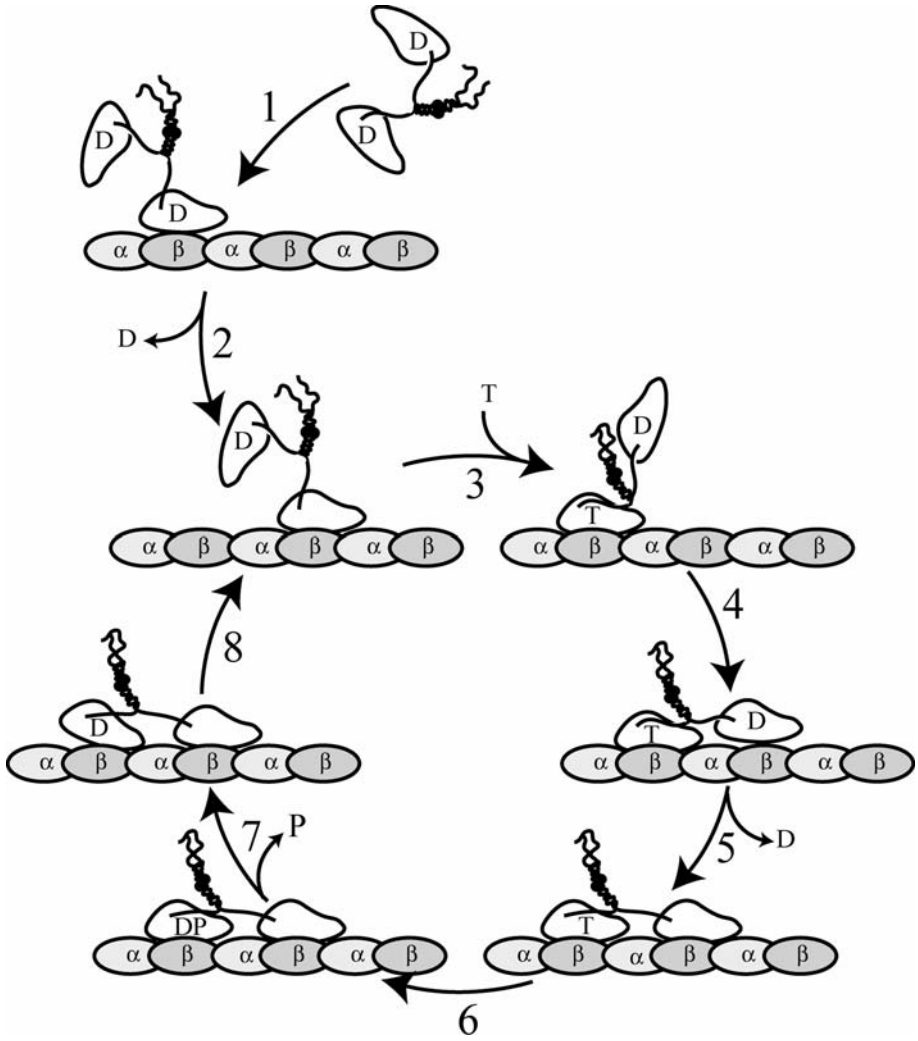

Fig. 1. Working model for the processive movement of Kinesin-I. Upon collision with the microtubule (1), the dimeric motor quickly looses the ADP molecule from the binding head (2), spatial constraints require binding of ATP and reorientation of the neck-linker (3) before the second head can bind to the next available binding site (4) and release ADP (5) to generate an internally strained doubly bound motor. It has been shown that the rearward strain on the leading head prevents ATP-binding, and we hypothesize that the forward strain on the rear head accelerates ATP-hydrolysis (6), which leads to rapid phosphate release (7) and dissociation of the ADP·kinesin-head (8) which allows the cycle to start again. Based on Lakamper and Meyhofer (2005).

ADP-state. Upon (productive) collision with the microtubule, binding triggers rapid ADP-release of one head, leading to the strongly bound nucleotide-free state, while the second head is unable to bind and thus retains its ADP. Only the rearrangement of the neck-linker upon ATP-binding on the so-called 'rear head' allows the second head to bind and to loose its ADP. Binding of the second head is believed to introduce intra-molecular strain, (i.e. backwards in the front head and forward in the rear head) and to (a) prevent ATP-binding to the front head in order to keep this head in the strongly bound state and (b) trigger ATP-hydrolysis and phosphate-release in the rear head. This latter process generates a weakly bound ADP-head which can subsequently dissociate and reorient such that the nucleotide-free head (now rear) can bind ATP and start the cycle again. The most prominent conformational changes accompanying nucleotide-changes in the kinesin head are (a) the immobilization of the otherwise highly mobile neck-linker upon ATP-binding (Rice *et al*., 1999) and (b) a rotation of the kinesin core associated with nucleotide-hydrolysis (Nitta *et al*., 2004). In the latter conformational change the kinesin motor-domain rotates about 20° counter-clockwise while increasing the affinity to the microtubule binding-interface. In order to understand how the structural rearrangements in the motor-domain drive kinesin's motility knowledge of the location and nature of the interaction-sites of kinesin and the microtubule is of considerable importance.

Interaction sites between kinesin and MTs

Where and how do the kinesin motors and MTs interact? The obvious interaction site is the classical nucleotide-dependent binding site (NDBS). In order to adapt to various cellular functions, kinesins not only

developed variations in the number, arrangement and function of classical binding sites (N-terminal and C-terminal motors, internal motors, as well as monomeric, homo- and hetero-dimeric and tetrameric motors), but also developed additional, nucleotide-independent MT-binding sites (NIBS) which aid in and/or modulate the interaction of the predominantly conserved kinesin–MT interaction site or serve other functions in the interaction with the microtubule. While the functional and structural distinction is usually rather clear, the influence of the NIBS can be so intimately intertwined in the mechanism of the NDBS that a distinction becomes less transparent. Interestingly, both NIBS and NDBS have a rather limited binding surface on the outer rim (Wendt *et al*., 2003) of the MT and might therefore compete with and influence each other or other microtubule associated proteins, like tau (Bonnet *et al*., 2001; Seitz *et al*., 2002). Of these NDBS and NIBS, the classical interaction between kinesin and the MT is best understood structurally.

NDBS

To identify residues of kinesin interacting with the tubulin-surface and *vice versa* several approaches have been used. EM-studies first established the interaction sites and showed that the binding sites for all kinesins examined so far (processive, non-processive, C-terminal, internal and N-terminal motors) appear to be very similar and primarily differ structurally in the more distal parts of the motor (see for example Hirose *et al*., 1995; Hoenger *et al*., 1995; Hoenger and Milligan, 1997; Hirose *et al*., 1999 and references cited therein). This work has been complemented by sophisticated biochemical and genetic approaches. Recently, for example, elegant combinations of cross-linking or foot-printing experiments with mass-spectroscopy or Western-blot analysis confirmed that the motor-domains of the kinesins studied so far share very similar interaction sites (Alonso *et al*., 1998). The MT binding site of conventional kinesin has been mapped in detail bio-chemically using cross-linking studies and alanine-scanning techniques (Song and Mandelkow, 1993; Tucker and Goldstein, 1997; Woehlke *et al*., 1997). Docking of the crystal-structure into 3D-electron-density maps of decorated microtubules confirmed the biochemical findings (Hoenger *et al*., 1995). The switch II cluster (α4/L11/α5) of conventional kinesin interacts with the helix H12 of β-tubulin while the region β5a/b forms partial contacts with both the β- and α-subunit (Song *et al*., 2001; Woehlke, 2001). Alanine-scanning approaches allowed determining the contribution of individual, surface-exposed kinesin-residues to the binding affinities. Interestingly, the most important residues are predominantly charged residues. Here substitution of negatively charged residues led to an increase in affinity (reduced K_MMT) while a substitution of positively charged residues found in close proximity led to a decrease in affinity (increased K_MMT). Interestingly, these residues are conserved (in both the superfamily and the Kinesin-I family) and cluster in two regions, loop 7 and loop 8 (loop 7 being in close proximity to the switch I region) and L11/α 4 (switch II-loop) together with L12/α5. While it is not completely understood if some of the substitutions are influencing affinity directly or indirectly, this study clearly highlights the importance of the structural elements and the predominantly electrostatic nature of the interaction (Figure 2). The identification of these regions is in agreement with findings from Nitta *et al*. who found crystallographic evidence that the helix α4,

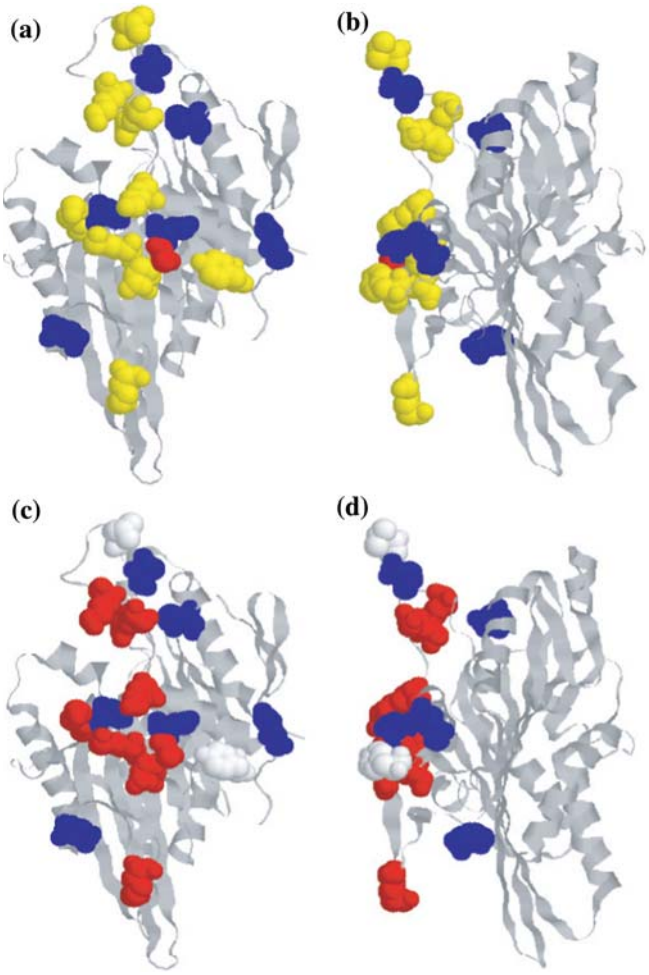

Fig. 2. The microtubule-binding site of Kinesin-I. (a) and (c) show the kinesin motor-domain after Kull (1996) with the nucleotide-binding site located on the back. (b) and (d) show a side-view of the molecule with the microtubule-binding side facing left. The microtubule plus-end would be oriented downwards. (a) and (b) replacement of residues with alanine result in affinity decreases (yellow) and increases (blue) in some residues. In the side-view it can be seen how these residues cluster on the microtubule-binding surface. (c) and (d) representation of the residues according to their charge. Residue L248 (top) and Tyr274 (middle) are in light gray, while positively charged residues are colored in red and negatively charged residues are blue. In the comparison between panels a/b and c/d it can be seen that replacement of positively charged residues leads to affinity decrease while replacement of negatively charged residues leads to an increased affinity.

which connects the MT-interacting loop11 and 12 and is nested between the α- and β-subunits of tubulin, tilts significantly upon ATP-binding (Nitta *et al*., 2004).

While the conformational changes in the kinesin motor domain upon MT-binding have received considerable attention there is comparatively little known about the active participation of the MT in kinesin-motility. It could be shown that MTs grown in the presence of the non-hydrolysable GTP-analog GMP-CPP are transported 30% faster by conventional kinesins (Vale *et al*., 1994). They were also less often running in curved paths than GDP-MTs. In agreement with the structural interpretation that GTP-protofilaments adopt a less curved conformations, they were shown to be less curved and more rigid. Interestingly, the in-solution ATPase-activity of kinesin on GMP-CPP MTs was unaffected (Vale *et al*., 1994). However, this result suggested that the tubulin within MTs might be able to adopt different conformations and that either the rigidity or the actual tubulin-conformation might influence kinesin motility. The fact that the microtubule can adopt a distinct conformation which alters the motile behavior of Kinesin-I can be seen as an initial indication that binding of the motor to the microtubule might not only trigger conformational changes in the motor (which for example lead to ADP-release) but also that possibly subsequent rearrangements in the kinesin-motor domain might force the microtubule-structure to rearrange.

In fact, changes in the tubulin structure upon kinesin binding were reported in cryo-EM-studies of ncd-decorated MTs (Hoenger and Milligan, 1997). But only recent, refined cryo-EM-studies with 15-mer MTs decorated with monomeric constructs of the fast fungal kinesin NcKin could reveal more details. 15-mer MTs, which display a right-handed super-twist, show distinct differences in electron-density-maps with and without decoration with motors (Krebs *et al*., 2004). Most prominently, binding of the motor to the β-subunit leads to continuous core electron-density along the protofilaments which is mainly brought about by structural changes in the α-subunit. Together with the observation that single, stabilized protofilaments can only be found in preparations that were incubated with motor molecules but never in plain MT samples, these findings are interpreted as a clear indication of axial stabilization of the protofilament. Concomitantly, a slight reduction of density on the outer rim of tubulin was observed, corresponding to the motor binding site. The stabilization of axial protofilaments is reminiscent of the increased gliding speed observed with GMP-CPP-MTs, (Vale *et al*., 1994). While it is unclear which mechanism is responsible for the increased speeds of GMP-CPP-MTs (the ATPase activity is apparently unchanged) and how the above structural and functional observations relate in detail, these observations argue for allosteric effects associated with kinesin binding, perhaps even cooperative axial effects over several tubulin subunits as inferred form the GTP-cap-hypothesis. Interestingly, a recent report provides some evidence in support of long-range cooperative binding of kinesin motors to microtubules, binding of kinesin coated beads to a MT led to increased binding frequency in the vicinity of the initial bead (Muto *et al*., 2005). This effect was only observed in the presence of ATP, but not AMP-PNP and ADP. Control experiments with hetero-dimeric motors consisting of a wt-kinesin and a hydrolysis-incompetent mutant (E236A) confirmed that ATP-hydrolysis is essential for the observed cooperative binding. It could further be shown that the cooperativity is slightly biased towards the (+)-end of the MT. While the data suggest cooperative effects, the idea of cooperative binding effects over a distance of 10–50 tubulin subunits is challenging. It remains to be determined how much energy is required and/or which conformational changes are necessary in the MT-lattice and the tubulin-monomers to allow for an increased binding rate.

NIBS

NIBS have first been described for the tail of the (−)-end directed and non-processive kinesin ncd (Karabay and Walker, 1999a,b, 2003). The N-terminal position of the NIBS of ncd is reminiscent of a conventional kinesin cargo-binding domain and has binding properties that are similar to microtubule associated proteins (MAPs, Wendt, 2003), suggesting that the MT-crosslinking effects reported *in vitro* reflects important functions of this mitotic motor *in vivo*, namely in the mitotic spindle (Karabay and Walker, 1999b). Other NIBS are located in the vicinity of the motor domain and have been shown to increase the affinity of the motor, but not the catalytic turnover. These affinity-increasing NIBS have been identified in N-terminal extensions of the BimC motor domain (Stock *et al*., 2003), but also in internal loops within the motor domain, the so called K-loop of Kif1A of the Unc104-family (Okada and Hirokawa, 2000). The K1A K-loop increases the affinity to the native MT so drastically, that monomeric Kif1A motors could be observed to diffuse in one dimension along the MT in a pseudo-processive manner (Okada and Hirokawa, 1999). A comparable mechanism has been suggested for the MT-depolymerizing kinesin MCAK (Ovechkina *et al*., 2002) while a similar mechanism based on electrostatic interactions of the dimeric neck of conventional kinesins with the microtubule (Thorn *et al*., 2000; Wang and Sheetz, 2000), has recently been challenged by others (Skiniotis *et al*., 2004) and us (Lakamper and Meyhofer, 2005, discussed in detail below). Attractive interactions of the stalk with the MT, however, have convincingly been shown to increase the affinity of the motor HsKID (Shiroguchi *et al*., 2003). Combinations of multiplied classical binding sites and NIBS can for example be found in the tetrameric motor BimC (Kashina *et al*., 1996; Shiroguchi *et al*., 2003).

There is little information about the actual structure and MT-interaction sites of NIBS located close to the motor domain. However, they all seem to share a predominantly electrostatic character. More information about these structures and their interaction with the MT might be inferred from the NIBS of the ncd-tail. Actually, the ncd-tail domain contains two NIBS, a weaker binding site at position 115–187 and a stronger (N-terminal residues 83–100, (Karabay and Walker, 1999a). Based on cryo-EM studies the ncd-fragment NT6, which contains both binding regions, displays no prominent globular domain and behaves rather like the filamentous MAP tau. However, the studies with NT6 revealed four sites of increased electron density on the outer rim of the microtubule (Wendt *et al.*, 2003).

Interestingly, the two MT binding sites of the tail of ncd interact with two regions of the most C-terminal structures of both the α- and β-subunit of tubulin: (1) the H12 on α-tubulin (ordered), (2) the C-terminus on α-tubulin (disordered, α-E-hook), (3) the C-terminal H12 on β-tubulin (ordered) and (4) the C-terminus on β-tubulin (disordered, β-E-hook (Karabay and Walker, 2003). Sites 1 and 3 together correspond to the classical binding site of conventional kinesin (Wendt, 2003). The transition H11–H12 loop and the H12-helices contain a significant number of negatively charged residues (E, acidic cluster), suggesting that the tail interactions are very much mediated by electrostatic interactions, which is in agreement with its MAP-like structural appearance and the reported salt sensitivity of NT6-interactions (Karabay and Walker, 2003). Interestingly, the finding of a MAP-like protein binding in this region suggests competition for binding of motors which has been described for conventional kinesin and MAPs by Seitz (2002). The other two other binding sites, 2 and 4, correspond to the estimated position of the so called E-hooks of tubulin (Karabay and Walker, 2003). These 12–18 AA long domains are not resolved in the crystal structures and are therefore interpreted to be highly flexible (Nogales *et al.*, 1998). The E-hooks show, however, a slight tendency to form helical structures *in vitro* (Jimenez *et al.*, 1999). The E-hooks can be removed from tubulin subunits by proteolytic treatment with subtilisin (Bhattacharyya *et al.*, 1985; Sackett *et al.*, 1985). The removal of the β-E-hook reduces not only the binding stochiometry from 4 to 3, but also drastically reduces the overall affinity of NT6 to the microtubule. Further removal of the α-E-hook leads to a binding stochiometry of two and lower affinities. The remaining two NT6 bind to the acidic cluster in the ordered H12. Furthermore, it could be shown that the interaction between the MT and NT6 is highly salt-sensitive (Karabay and Walker, 2003). These findings of NT6-interactions with the E-hook provide a structural basis for the electrostatic interactions of other NIBS and pinpoint the importance of the E-hook.

Specific roles for the E-hook in microtubule–Kinesin-I interactions

The E-hook has mainly been described in the context of NIBS-mediated binding. For example, the pseudo-processive, one dimensional diffusion of monomeric Kif1A constructs on native microtubules (Okada and Hirokawa, 1999) is abolished by either removing the highly positively charged K-loop-insertion in the motor or by removal of the E-hook (Nitta *et al.*, 2004; Okada and Hirokawa, 2000). This and the high salt-sensitivity suggest strong electrostatic interactions, similar to what has been described for the ncd-tail. Equivalent experimental approaches have been performed to asses the role of the E-hook for KID (Shiroguchi *et al.*, 2003), MCAK (Ovechkina *et al.*, 2002), BimC (Stock *et al.*, 2003) and conventional kinesin. While the results for KIF, KID, MCAK and BimC suggested that the main role of the E-hook is an increase in the kinesin–MT affinity (in agreement with electrostatic interactions), the removal of the E-hook led to surprisingly different effects in conventional kinesins, namely a reduction in speed and processivity (Wang and Sheetz, 2000). On the basis of elegant experimental approaches, an electrostatic interaction of the E-hook with the dimeric neck of conventional kinesin was proposed to be the structural basis for the observed E-hook function. (Thorn *et al.*, 2000).

In contrast to this interpretation, it has been shown in earlier experiments that both E-hooks efficiently cross-link directly to the kinesin motor domains (Tucker and Goldstein, 1997), although there is a preference for the β-subunit as demonstrated in another study (Song and Mandelkow, 1993). The cross-links to the motor-domain are very likely to attach to regions that correspond well with the interaction sites obtained by alanine scanning methods of kinesin (Woehlke *et al.*, 1997). Removal of the E-hooks and addition of E-hook peptides reduced cross-linking efficiency (Tucker and Goldstein, 1997). Two more recent lines of evidence further support the hypothesis that the E-hook is not just passively associating with NIBSs of the motor, but directly interacting with the core motor domain to modify its kinetics and subsequently its processivity (Skiniotis *et al.*, 2004; Lakamper and Meyhofer, 2005). On one hand, cryo-EM studies of the native and digested MTs decorated with minimal motor-domain under different nucleotide conditions revealed that much larger fractions of motor bind to the digested MTs under ADP conditions. Further kinetic studies provided evidence of a previously unknown ADP-state, which is trapped on the digested microtubule (Skiniotis *et al.*, 2004). Consequently, the E-hook has important functions for kinesins ADP and/or binding kinetics. On the other hand, using a different approach with fungal conventional kinesin NcKin, our group provided complementary evidence. We observed reduced processivity and speed on digested microtubules in single molecule fluorescence

assays, although the neck of NcKin does not carry positive charges. This strongly argues against the proposed anchoring interaction of the neck of conventional kinesin and the E-hook. Together with kinetic experiments, multiple molecule gliding assays and single molecule trapping experiments our results strongly argue for a direct interaction between the E-hook and the motor domain. From the measurement of the stall force we concluded that the removal of the E-hook does not influence strongly bound states (ATP and NT-free) of NcKin, but ADP-states. Binding studies reveal a similar increase in the fraction of NcKin minimal motors bound to the digested MT, suggesting a common mechanistic function of the E-hook for fungal and animal conventional kinesins (Lakamper and Meyhofer, 2005). The exact mechanism of how the E-hook interacts with the motor remains to be elucidated through further cross-linking and mutational studies. These results stress the complex role of microtubules in kinesin motility and serve as a clear example in which the distinction between NDBS and NIBS reaches its limitations, as the direct influence of structures outside the classical interaction sites on the nucleotide-kinetics becomes evident. Even more interestingly, the tubulin structure which influences kinesin motility most, the E-hook, is the mayor site of differences between tubulin iso-forms (Sullivan and Cleveland, 1986) and subject of extensive post-translational modifications (Westermann and Weber, 2003).

Post-translational modifications on the E-hook of tubulin and their relevance for kinesin motility and cellular function

Diverse functions of MTs in cell motility and cell architecture are likely to be reflected in tubulin-diversity. As observed for other proteins, the cell uses two mechanisms to provide tubulin variants. Expression of αβ-tubulin iso-types/iso-forms and post-translationally modified tubulin. With the exception of acetylation of α-tubulin at the inside of the microtubule, the predominant sources of tubulin-diversity are the mostly disordered C-termini (CTT, E-hooks) on the outer-rim of the microtubule (Wendt *et al*., 2003; Westermann and Weber, 2003). As described above, these regions coincide with the mayor sites of kinesin interactions with the microtubule. A brief summary of the possible (regulatory) functions of kinesin's post-translational modifications and relations to kinesin is given in Table 1.

While distinct tubulin iso-forms are responsible for very specific functions, like γ-tubulin in the MTOC, most minor variations in the C-termini of the predominant α- and β-tubulin monomers (iso-types) seem to be interchangeable (Luduena, 1998). Nevertheless, structurally similar iso-forms may be functionally different (Hutchens *et al*., 1997). Reports of axoneme-specific β-tubulin motifs and β-isoform-specific MT-architecture (Raff *et al*., 1997; Nielsen *et al*., 2001) were challenged recently by experiments in which α- and β-CTTs were deleted, interchanged and combined in order to test for the rescue of lethal heterokaryons of *T. thermophila* (Duan and Gorovsky, 2002). Apart form the notion that the tail (CTT) has essential functions not associated with post-translational modifications, this study showed that *T. thermophila* requires sufficiently extended, but interchangeable regions of post-translational modifications on the CTT of at least one tubulin subunit, possibly cross-talking between the α- and β-subunit through the E-hooks Redeker *et al*., 2005).

The interpretation that tubulin-distribution in MT and axonemes is reflected in, possibly even caused by, the type of modification in the tail is supported by observations in sea urchin sperm axonemes and Chlamydomonas flagellar axonemes: (1) the A-tubules of

Table 1. Overview over the post-translational tubulin modifications and the proposed relation to kinesin motors

Modification	Tubulin	Enzymes	Possible function	Proposed relation to kinesin
Tyrosination/ detyrosination	Only α E-hook	TTL	IF-network Differentiation (?)	Kinesin cross-links IFs to MTs
Δ2-Tubulin	Only α E-hook	Specific TTCP (?)	Tubulin maturation Glycylation marker (?)	Role of tyrosine in kinesin-motility (?)
Glycylation	α and β E-hook	?	Axonemal organization Cytokinesis	Kinesin-mediated IFT affected (?) MT-Severing-enzyme transport affected (?)
Glutamylation	α and β E-hook	TTL-like proteins Nek	Centriole maturation Axonemal organization MAP-binding	Kinesin-mediated IFT affected (?) Interference with kinesin-mediated transport
Acetylation	Only α N-Terminal region	HDAC6, SIRT2	Cell motility MAP-binding	Structural effects (?) Interference with kinesin-mediate transport
Palmitoylation	α, β (?)	?	Membrane interactions (?) Microtubule positioning	None so far
Phosphorylation	β, α (?)	?	Differentiation (?)	None so far

TTL, tubulin–tyrosine–ligase; IF, intermediate filament; TTCP, tubulin tyrosin caboxy-peptidase, Nek, NIMA(never in mitosis gene)-related kinase; IFT, intraflagellar transport; MAP, microtubule associated protein; HDAC6, histone deacetylase 6; SIRT2, Sir2 homolog.

outer axoneme doublets are mostly unmodified (Tyr-tubulin without glytamylation or glycylation) while the B-tubules are glutamylated and detyrosinated (Glu-tubulin, see below) (Multigner *et al*., 1996; Johnson, 1998; Mencarelli *et al*., 2000). (2) The axoneme architecture leads to an end-region enriched in tyrosinated, non-modified tubulin. Interestingly, the level of glutamylation and glygcylation decreases towards the end, possibly suggesting modification to be an age marker (Bre *et al*., 1996; Huitorel *et al*., 2002). Similarly, while detyrosination does not affect MT-stability, it might serve as an indicator of how long a MT has been assembled (Raybin and Flavin, 1977b; Webster *et al*., 1990; Gurland and Gundersen, 1995).

Post-translational modifications of the CTT have been described mainly for cells and organisms containing axonemal organelles, but also take place in non-axoneme containing cells. While acetylation of α-tubulin on residue 40 is likely to have effects on kinesin motility, this is not discussed in this review as it is not directly linked to the E-hook. The three mayor classes of post-translational modifications of the E-hook which have implications for kinesin motility and function are tyrosination, and glutamylation and glycylations. Palmitylation and Phosphorylation of tubulin has been observed for α- and β- tubulin, respectively, but have not been studied extensively yet.

Tyrosination

While glycylation and glutamylation occurs in both α- and β-tubulin, only α-tubulin is post-translationally modified by addition or removal of the most C-terminal tyrosine residue (Westermann and Weber, 2003). The C-terminal tyrosine encoded in most isolated mRNAs for α-tubulin, is removed by a currently unidentified, maybe non-specific enzyme activity, tubulin tyrosine carboxyl-peptidase, to generate so called Glu-tubulin (TTCP, (Arce *et al*., 1975; Raybin and Flavin, 1977a,b; Argarana *et al*., 1978; Argarana *et al*., 1980). In an ATP-dependent process, mediated by tubulin tyrosine ligase (TTL, (Murofushi, 1980; Ersfeld *et al*., 1993; Idriss, 2000) can restore the initial C-terminus. Loss of the C-terminal Glutamate of Glu-tubulin yields Δ2-tubulin, which cannot be tyrosinated by TTL anymore (Paturle-Lafanechere, 1991). It is so far unknown if this deglutamylation is mediated by specific or unspecific proteases. Detyrosination, on the other hand, can be prevented by TTL-mediated incorporation of 3-nitro-tyrosine, which is generated *in vivo* in response to nitric-oxide (Eiserich *et al*., 1999; Kalisz *et al*., 2000). Similarly, TTL catalyzes the irreversible incorporation of Aza-tyrosine into the C-terminus (Purro *et al*., 2003).

Although the tyrosination cycle of tubulin occurs only at the α-tubulin and kinesin binding is predominantly mediated through contacts with the β-tubulin, influences of this type of modification on the interaction between kinesin and tubulin have been reported. Kinesin was shown to be necessary for the extended distribution of IFs in the cell, as injection of anti-bodies against KHC collapsed the IF network to perinuclear locations (Gyoeva and Gelfand, 1991). Furthermore, it could be shown that vimentin-IFs localize preferentially along detyrosinated, Glu-MTs *in vivo* (Gurland and Gundersen, 1995). Together, these data suggest kinesin to be a preferential cross-linker between vimentin-IFs and Glu-tubulin, not tyr-tubulin. In fact binding studies *in vitro* and *in vivo* could confirm that tissue-purified kinesin binds to Glu-MTs with a 2.8-fold higher affinity (Liao and Gundersen, 1998) and that micro-injection of monomeric Glu-tubulin in cells induces the collapse if the extended IF-network most probably due to kinesin depletion from stable Glu-MTs (Kreitzer *et al*., 1999).

The collapse of the IF network in response to a kinesin depletion has also been observed in a complex chain of events caused by the over-expression of tau in astrocytes (Yoshiyama *et al*., 2003), high levels of tau led to sharp decrease in stable Glu-MTs accompanied by an increase in tubulin synthesis and accumulation of Tyr-MTs. An observed concomitant decrease of kinesin levels led to the collapse of the IF network and progressive disruption of kinesin-mediated transport. In this study, over-expression of tau ultimately led to atrophy and non-apoptotic cell death. While other studies have linked interference of tau and other structural MAPS with kinesins binding to the MT to the development of Alzheimers (Ebneth *et al*., 1998; Seitz *et al*., 2002), this study stresses the role of kinesin and tubulin-tyrosination as a regulatory process (Yoshiyama *et al*., 2003). Interestingly, several studies reveal that TTL-activity is reduced or absent in breast cancer (Mialhe *et al*., 2001), and cell-lines with low TTL-activity frequently induce tumor-formation (Lafanechere *et al*., 1998) due to increased levels of Glu-tubulin. The specific incorporation of Aza-tyrosine could counterbalance of c-Ha-ras mediated tumor induction (Purro *et al*., 2003). TLL-mediated Nitro-tyrosine incorporation (Kalisz *et al*., 2000) has been linked to MT dysfunction in lung carcinoma cells (Eiserich *et al*., 1999) but also to the blocking of early myogenic differentiation L6 Myoblasts (Chang *et al*., 2002), stressing the possible role of properly tyrosinated MTs in cell maintenance.

There is little structural information available on the cause of the increased affinity of kinesin to Glu-tubulin. The observation that monomeric heads of conventional kinesins bind in ADP-trapped state to MTs without E-hooks (Skiniotis *et al*., 2004), might provide interesting clues, but little specific information, as the complete E-hook includes other possible sites of post-translational modifications. However, an unexpected increase of ATPase-activity of truncated, monomeric constructs of the fungal conventional kinesin NcKin, NK378 and NK383, on digested microtubules might be connected to tubulin-tyrosination (Lakamper and Meyhofer, 2005), the activity of these constructs on undigested

tissue-purified MTs is tightly controlled by a highly conserved, inhibitory tyrosine residue (Y362) that is buried in the neck-coiled coil in functional dimeric constructs (Schafer *et al*., 2003; Bathe *et al*., 2005). The specific site and mechanism of inhibition is so far unknown, but it is conceivable that the terminal tyrosine of the E-hooks of α-tubulin plays a role through unknown mechanisms.

Glycylation

The reversible addition of 1–40 glycine residue to one or more specific glutamate residue in the flexible E-hook region of both α- and β-tubulin was first described in Paramecium (Redeker *et al*., 1994; Rudiger *et al*., 1995; Mary *et al*., 1996; Plessmann and Weber, 1997). It is a post-translational mechanism that is unique to tubulin and occurs only in cell types that contain either cilia or flagella and here prominently in the axonemal microtubules (Levilliers *et al*., 1995; Bre *et al*., 1998; Xia *et al*., 2000; Westermann and Weber, 2003). The enzymes catalyzing the addition of the glycines have not been isolated to date.

The possibility to produce heterokaryons in the binucleate ciliate *Tetrahymena* allowed to study combinations of genetically modified α- and β-tubulin genes (Redeker *et al*., 2005). Interestingly, the three-glutamylation sites identified in α-tubulin are not essential, whereas similar sites in β-tubulin are essential, but lethal mutants could be rescued by co-transformation with α-tubulin-genes that carried a full length β-tubulin-E-hook. Unexpectedly, some mutations in the β-glycylation sites, affected poly-modifications in the α-tubulin subunit, suggesting a cross-talk between the two subunits of the tubulin dimer through an unknown mechanism (Redeker *et al*., 2005). The compromised but viable polyglycylation mutant $\beta EDDDD_{440}$ grows slow, and has severe effects on the axonemal architecture, most mutants form defective axonemes lacking the central pair of A-tubules. Furthermore, the mutants display reduced motility and are defective in cytokinesis (Xia *et al*., 2000; Thazhath *et al*., 2002), possibly suggesting effects on the interaction between the mutated E-hooks with dynein and/or kinesin. However, so far the observed effects have not been convincingly linked to any kinesin, although changes in intra-flagellar transport have been described that could be linked to kinesin-II (Brown, 1999).

Glutamylation

While polyglycylations have been extensively studied using *Tetrahymena* heterokaryons, the effect of mutations in the E-hook on polyglutamylation has not yet been studied as extensive using this method. However, polyglutamylation of α-tubulin seems to play a key role in flagellar motility (Gagnon *et al*., 1996; Million *et al*., 1999). Polygutamylation of multiple neighboring glutamate residues of α- and β-tubulin is described for many organisms and isoforms (Kann *et al*., 2003). Very recently the tubulin polyglutamylase could be identified as a TTL-domain enzyme (Janke, 2005). Particularly interesting in the context of kinesin is the observation of prominent polyglutamylation of MTs in neuronal axons, while polyglutamylation in non-neuronal cells is mainly detected in the centrioles, the mitotic spindle and midbody, as well as the primary cilium (Bobinnec *et al*., 1998).

In contrast to the electrostatically neutral polyglycylation, polyglutamylation adds up to 20 highly negatively charged glutamate-residues to the already negatively charged E-hooks of both α- and β-tubulin. It could be shown that the affinity of structural MAPs, like tau and MAP2 (Bonnet *et al*., 2001), as well as kinesin increases progressively by addition of 1–3 glutamate residues, while further glutamate-addition gradually decreases their affinity (Boucher *et al*., 1994; Larcher *et al*., 1996). Through the binding of different MAPs to distinct MT-binding sites (similar to the different binding sites of the ncd- tail) it seems to be possible to selectively recruit MAPs depending on the site's ability to be polyglutamylated (Bonnet *et al*., 2001). An interesting study of the low glutamylation levels of arctic fish tubulin (Redeker *et al*., 2004) infers very important information about microtubule assembly at low temperatures and might explain the low critical concentrations of subtilisin-treated tubulin (Sackett *et al*., 1985). However, it also stresses the importance of robust double glutamylation of a specific isotype (β4(III)) for MAP and motor binding.

Conclusions and outlook

The literature of tubulin and microtubules contains a significant number of publications that hint at important roles of the structure and/or post-translational modification of α-and β-tubulin. However, a detailed, mechanistic picture of how kinesin's motility and function within the cellular context are modulated is only slowly emerging. One reason for the lack of rapid progress is the difficulty to obtain tubulin-preparations of well-defined post-translational modifications. Therefore, conformational changes within the microtubule might be overlaid or influenced by effects caused by post-translational modifications. The rather crude (and incomplete) approach to remove most sites of post-translational modification has served well to answer important initial questions, but further understanding is limited. In order to understand the molecular mechanisms that regulate the MT–kinesin interaction it is highly desirable to obtain genetically modified tubulin-mutants of defined modification status. Viable *Tetrahymena* heterokaryons might provide access to more defined tubulin preparations and promise to be helpful in determining if the cell is able to regulate cellular transport and the activity of different kinesins at the level of the microtubule. At the same time these mutants allow to examine the effects on the cellular

function and viability (Gaertig *et al*., 1993; 1995). Such investigations should be complemented by detailed biophysical single molecule studies *in vitro* and *in vivo*.

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