

The kinetic mechanism of kinesin

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The chemical kinetic mechanism of kinesin (K) is considered by using a consensus scheme incorporating biochemically defined open, closed and trapped states. In the absence of microtubules, the dominant species is a trapped $K\bullet ADP$ state, which is defined by its ultra-slow release of ADP (off rate, $k_{off} \approx 0.002\text{ s}^{-1}$) and weak microtubule binding (dissociation constant, $K_d \approx 10\text{--}20\ \mu\text{M}$). Once bound, this trapped state equilibrates with a strongly binding open state that rapidly releases ADP ($k_{off} \approx 300\text{ s}^{-1}$). After ADP release, $Mg\bullet ATP$ binds (on rate, $k_{on} \approx 2\ \mu\text{M}^{-1}\text{ s}^{-1}$) driving formation of a closed state that is defined by hydrolysis competence and by strong binding to microtubules. Hydrolysis ($k_{hyd} \approx 100\text{--}300\text{ s}^{-1}$) and phosphate release ($k_{off} > 100\text{ s}^{-1}$) both occur in this microtubule-bound closed state. Phosphate release acts as a gate that controls reversion to the trapped $K\bullet ADP$ state, which detaches from the microtubule, completing the cycle.

Kinesin is a molecular walking machine that is driven by ATP turnover. So how do the chemical steps of ATP turnover drive the mechanical steps of the motor? Here I discuss recent progress towards answering this question by considering a consensus kinetic model and by assessing which aspects are well supported by evidence and which are less certain.

The first studies of the kinesin kinetic mechanism were done on purified brain kinesin (now called Kif5b, kinesin-1 or KHC), and this molecule remains the best-studied member of the kinesin superfamily. It consists of two identical head domains connected to a coiled-coil tail, and a pair of light chains that bind to the distal (carboxy-terminal) end of the tail. Early work on kinesin was difficult, heroic even, because of inhomogeneities in the sample preparations. Nevertheless, the early studies established several important points: namely, microtubules increase the rate of ATP turnover; the non-hydrolysable analogue AMPPNP, which is presumed to mimic ATP, induces a tightly bound (stable) microtubule–kinesin complex; and ADP release is the rate-limiting step of ATP turnover in the absence of microtubules [1]. Intact kinesin was found to undergo a 9S-to-6S conformational transition (a folding and unfolding of the tail) that inhibits the ATPase, and subsequent work has used bacterially expressed and purified kinesin, which is usually truncated to either a single head domain or pairs of heads joined to a truncated tail.

An outline of the kinetic mechanism for one kinesin head [2] is shown in Figure 1. This scheme has similarities to the scheme for myosin [3] in that ATP turnover serves cyclically to shift the conformational equilibrium among strong, weak and detached binding states of the motor. Strong states are stably attached to the microtubule on the timescale of interest; weak states are not. Below, I discuss separately each step in the scheme.

ATP binding

To measure $Mg\bullet ATP$ binding to kinesin, it is first necessary to strip out the $Mg\bullet ADP$ that, in the absence of microtubules, is trapped in the kinesin active site ($k_{off} \approx 0.002\text{ s}^{-1}$ for wild-type dimers [4]). Gel filtration in the presence of EDTA or treatment with apyrase removes $Mg\bullet ADP$, but the resulting apo-kinesin heads denature in a few minutes unless stabilizing agents are added [5]. For several kinesins, it has proved so far impossible to prevent the apo enzyme from denaturing [6–8]. For single apo-kinesin heads in the absence of microtubules, the rate of ATP binding is $\sim 4\ \mu\text{M}^{-1}\text{ s}^{-1}$ [9,10].

The ratio of the binding and unbinding rate constants is the K_d for ATP dissociation from the kinesin–microtubule complex ($\sim 75\ \mu\text{M}$), which approximates the Michaelis constant (K_m) for ATP as a substrate of the microtubule-activated ATPase ($\sim 50\ \mu\text{M}$ [11]; note, however, that this value is highly dependent on ionic strength). Kinesin dimers moving at low load take $\sim 50\text{--}200$ steps per second; each step requires one molecule of ATP [12,13] and the two heads step alternately [14], implying an ATPase cycle rate per head of $\sim 50\text{ s}^{-1}$. Measured cycle rates for isolated single heads approximate this value [15].

The complex of apo-kinesin heads with microtubules is often called ‘rigor’ after the equivalent state of myosin in muscle. The rigor complex is extremely stable, with an unbinding rate constant of $\sim 0.002\text{ s}^{-1}$ [16]. ATP binds to the rigor complex at a rate of $2\text{--}4\ \mu\text{M}^{-1}\text{ s}^{-1}$ [9,10,17]. The rate of ATP unbinding is $\sim 150\text{ s}^{-1}$. Importantly, ATP and ADP bind and dissociate at similar rates, such that ADP can be a significant competitive inhibitor of the kinesin ATPase.

Mg^{2+} is crucial for stabilizing nucleotide binding, and thus is also crucial for ATPase activity. Ca^{2+} cannot substitute for Mg^{2+} [18]. The weight of the evidence indicates that $K\bullet ATP$ binds strongly to microtubules. By contrast, myosin $\bullet ATP$ dissociates rapidly from actin [19].

Binding of other nucleotides

AMPPNP is a transition state analogue that is hydrolysed extremely slowly (0.00004 s^{-1}) [20]. The $K\bullet AMPNP$ complex is assumed to mimic $K\bullet ATP$ and binds strongly (stably) to microtubules with a submicromolar K_d [21] and

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Available online 6 May 2004

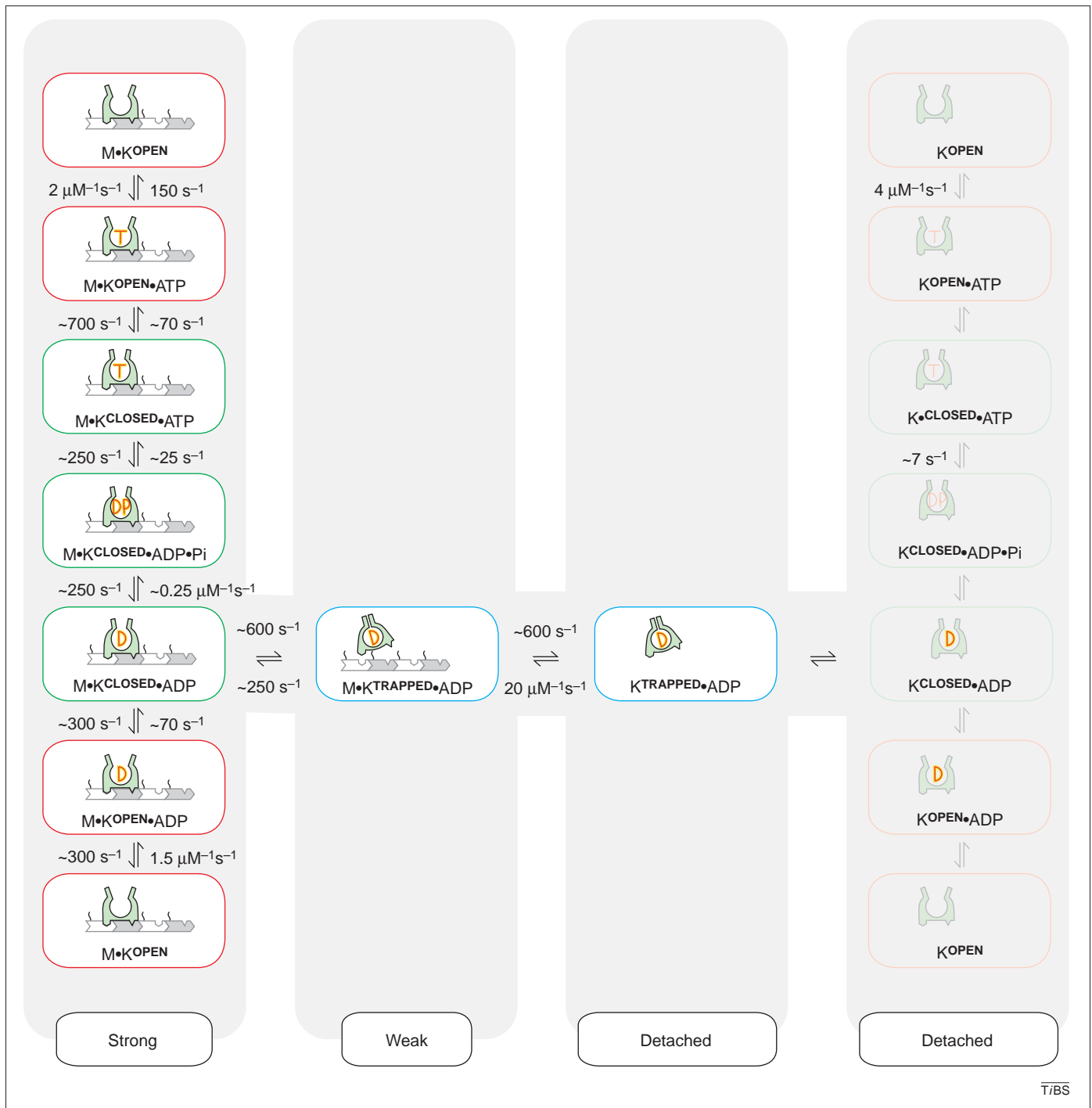


Figure 1. Model of the kinetic mechanism for one kinesin head. In this model, binding of nucleotide is followed by a conformational change, the open-to-closed transition, which can be driven by either ATP (T) binding or ADP (D) binding. Binding and unbinding of microtubules (M) occurs dominantly via an ADP-bound kinesin state ($K^{TRAPPED} \cdot ADP$). Rate constants for nucleotide binding and dissociation are consistent with literature values [5,10,44]. The rate constant for complex formation between $K^{TRAPPED} \cdot ADP$ and M is close to the diffusion limit [76]. Values for the other rate constants are estimates, as indicated by the tildes (~), and were generated by simulating the scheme using the following constraints: ADP-induced dissociation of $M \cdot K$, $40 s^{-1}$; ATP-induced dissociation of $M \cdot K$, $70 s^{-1}$ [5,58]; dissociation constant (K_d) for ATP binding to $M \cdot K$, $\sim 75 \mu M$ [9]; K_d for ADP binding to $M \cdot K$, $\sim 120 \mu M$; K_d for $K \cdot ADP$ binding to M, $20\text{--}30 \mu M$ [21]; microtubule-activated transient release of ADP from $K \cdot ADP$, $> 100 s^{-1}$ [50]; and transient ADP release from $K^{OPEN} \cdot ADP$, $\sim 300 s^{-1}$ [5,36]. Turnover on dissociated heads can occur but is shown dimmed to indicate that it is a minor pathway owing to the ultra-low rate of ADP release from the $K^{TRAPPED} \cdot ADP$ state ($0.02 s^{-1}$).

a k_{off} of $\sim 0.0025 s^{-1}$ [22]. AMPPNP competes relatively poorly with ATP ($K_d \approx 1 mM$ [10,21]), but millimolar concentrations of AMPPNP drive the formation of a stable microtubule– $K \cdot AMPPNP$ complex; indeed, this was the key to the original purification of kinesin as a complex with microtubules from cell extracts [1].

The slowly hydrolysed analogue ATP- γS causes

tighter steady-state binding of kinesin to microtubules than does authentic ATP [10], strengthening the case that the pre-hydrolysis complex with $Mg \cdot ATP$ itself binds tightly to microtubules. Transient rates of ATP- γS hydrolysis and thiophosphate release have not been reported. GTP is a substrate for kinesin and drives microtubule sliding at similar rates to ATP; by

contrast, other nucleoside triphosphates are poorer substrates [18,23].

Competitive inhibition of ATP binding by ADP

Mg•ADP is a competitive inhibitor of Mg•ATP binding to kinesin [18], with an inhibition constant (K_i) of $\sim 150 \mu\text{M}$. Mg•ADP tends to dissociate kinesin from microtubules [24]. It binds to microtubule-attached single heads at a rate of around $1.5 \mu\text{M}^{-1} \text{s}^{-1}$ [10]. When added to single-molecule motility assays, Mg•ADP reduces both the velocity and run length of walking dimers [22].

ATP-driven open-to-closed transition

The scheme in Figure 1 includes a conformational change that follows ATP binding and precedes hydrolysis. Kinesin, in common with myosin, is thought to undergo an ATP-induced conformational transition in which the switch regions that flank the active site shift between open and closed states [25]. The closed state is catalytically competent, whereas the open state is not. By analogy with myosin [25], closure enables a salt bridge to form between the switch 1 (SSRSH) and switch 2 (LAGSE) regions that sandwich the nucleotide. This salt bridge is crucial for rapid hydrolysis: it is involved in positioning the lytic water, and mutants in which either the arginine or the glutamic acid is changed to an alanine have a hydrolysis rate of 1% of wild type [26,27,28]. Including this transition in Figure 1 solves a problem raised by Schief and Howard [29], namely, that the low affinity and high apparent on-rate of ATP implies that ATP binding is followed by a very rapid process. Schief and Howard suggested this was hydrolysis itself, but it could be a conformational change that enables hydrolysis, as in Figure 1.

Several lines of evidence point directly to a conformational change occurring before hydrolysis. ATP binding to microtubule-attached single heads saturates at $\sim 700 \text{s}^{-1}$, whereas hydrolysis itself is slower. An open-to-closed transition is a good candidate for this step. In myosin, a similar transition is sensed by a fluorescence enhancement of an intrinsic tryptophan, is rapidly reversible and occurs at $\sim 1000 \text{s}^{-1}$ [30]. For kinesin, the non-hydrolysable analogue AMPPNP produces a conformational change, as shown by the fact that AMPPNP binding to the rear (trail) head of a kinesin dimer triggers ADP release from the tethered (lead) head, and by a distinct proteolytic fingerprint [31]. Rosenfeld and colleagues [32] detected a further conformational change before hydrolysis using enhanced fluorescence resonance energy transfer (FRET) between microtubule tryptophans and an AEDANS probe on the neck linker. At present, it is not clear whether ATP turnover is paused while this latter rearrangement completes, therefore, Figure 1 does not include it as a separate kinetic step.

Hydrolysis

In the absence of microtubules, hydrolysis by single heads is $\sim 7 \text{s}^{-1}$ [5]. Microtubule binding accelerates this to $200\text{--}300 \text{s}^{-1}$ [5]. This is one major difference between myosin and kinesin cycles: for myosin, hydrolysis occurs in a closed state while the heads are detached from actin; for kinesin, hydrolysis in the closed state occurs while the

head is attached to a microtubule. The acceleration of hydrolysis by microtubules might be due to allosteric stabilization by microtubules of the catalytically active kinesin conformation, or to direct binding of microtubules to the switch regions [33], or, potentially, to tubulin residues directly contributing to the active site [34]. The detailed mechanism of hydrolysis is uncertain but is predicted to depend on a water molecule being positioned for in-line attack of the $\beta\text{--}\gamma$ phosphate bond. By analogy to myosin [35], a conserved switch 2 glycine (LAGSE) and switch 1 serine (SSRSH) probably hydrogen bond to the γ phosphate, while another switch 2 serine (SSRSH) and a conserved threonine (GESGSKT) coordinate the Mg ion.

Early oxygen exchange work on native kinesin [4] showed that, in contrast to myosin, the backwards flux through the hydrolysis step was low, such that hydrolysis is effectively irreversible. Work in progress (Hackney, personal communication) confirms that flux is low, but finite.

Phosphate release

It is clear that for kinesin single heads, phosphate is released before MgADP simply because single heads in the absence of microtubules trap MgADP but not phosphate. Based on the microtubule activated cycle rate and the measured rate constants of other steps, the transient rate of phosphate release from a microtubule-attached head must be $> 100 \text{s}^{-1}$ [36]. Phosphate release can be measured directly by using the phosphate-sensing protein developed by Webb [37]. Where this has been done [37,38], the measured transient rate of phosphate release is close to the hydrolysis rate, suggesting that it might be limited by the preceding hydrolysis.

Does phosphate release take place on or off the microtubule? In myosins, the large drop in free energy associated with phosphate release drives the principal force-generating conformational change. For kinesins, the $\text{K}\cdot\text{ADP}\cdot\text{P}_i$ analogues $\text{K}\cdot\text{ADP}\cdot\text{AlF}_4$ and $\text{K}\cdot\text{ADP}\cdot\text{BeF}_x$ have a K_d in the low micromolar range that is clearly smaller than the $10\text{--}20 \mu\text{M}$ typically measured for $\text{K}\cdot\text{ADP}$ [5,21,39]. It is possible, however, that these analogues do not accurately mimic the real $\text{K}\cdot\text{ADP}\cdot\text{P}_i$ state, but instead mimic a transition state complex in which the $\beta\text{--}\gamma$ phosphate bond is energised but intact. If so, it is still possible that the $\text{K}\cdot\text{ADP}\cdot\text{P}_i$ state is a dissociating state. Nevertheless, the simplest interpretation of the data is that $\text{K}\cdot\text{ADP}\cdot\text{P}_i$ binds strongly to the microtubule. Further weight is lent to this view by recent experiments on a point mutant that is blocked in detachment [38]. Phosphate release takes place rapidly from the trail head of mutant dimers, indicating that, in this mutant at least, detachment of the kinesin head from the microtubule is not a prerequisite for phosphate release.

Binding of phosphate to microtubule-bound $\text{K}\cdot\text{ADP}$ heads is relatively weak (K_d is in the low millimolar range [40]), as it must be if phosphate release is to occur in cells at the usual millimolar concentrations of phosphate found in the cytoplasm. In motility assays, added phosphate acts as a competitive inhibitor of ATP binding [40]. Added phosphate inhibits ATP- or ADP-induced unbinding of

kinesin from microtubules [2], which is again consistent with microtubule–K•ADP•P_i being a strongly bound state.

A key difference between myosin and kinesin is that the detached state of kinesin traps product ADP, whereas that of myosin traps both ADP and phosphate. The rate of phosphate release from kinesin monomers is not greatly affected by microtubule binding. Superficially, this observation suggests that phosphate release does nothing useful in terms of force generation in the kinesin cycle. The preponderance of the evidence indicates, however, that K•ADP•P_i is strongly bound and therefore that phosphate release occurs from a microtubule-attached closed state. A large change in free energy associated with phosphate release could thus be driving a power stroke, as it does for myosin, in addition to controlling detachment by regulating access to the weak binding K^{TRAPPED}•ADP state.

Microtubule-activated ADP release

The rate of ADP release from wild-type dimers in the absence of microtubules is $\sim 0.002\text{ s}^{-1}$ and rate limiting [4]. In the presence of microtubules, ADP release is accelerated $\sim 10^4$ -fold. Hackney [41] made the key discovery that ADP is released sequentially from dimers. He found that when K•ADP dimers are mixed with microtubules, only one of the two trapped ADPs is released. Release of the second ADP depends on binding of an ATP 'chase' to the first head. Subsequent work using the fluorescent analogue mantADP, which is released at almost the same rate as ADP [42,43], has shown that other nucleotides can chase but are less effective (ADP, $\sim 6\text{ s}^{-1}$; AMPPNP, $\sim 30\text{ s}^{-1}$; ATP- γ S, $\sim 30\text{ s}^{-1}$) [10,36,44,45].

The rate constant for ATP-induced ADP release is a key number and has been surprisingly difficult to pin down (discussed in Ref. [36]). It is important because a value close to the cycle rate would indicate that this step is rate limiting [46]. Reported values vary between 60 s^{-1} and 300 s^{-1} . The rate of ATP-induced ADP release from Kif5b dimers depends strongly on ionic strength [43,47,48] and on the electrostatics of the kinesin–microtubule interaction, which could account for at least some of the spread in the data. Insertion of extra lysines into the K-loop adjacent to loop 12 of monomeric Kif1a strengthens the predominantly electrostatic binding of K^{TRAPPED}•ADP to microtubules [49]. Inserting lysines into the equivalent loop 12 of Kif5b dimers stabilizes the microtubule–K^{TRAPPED}•ADP state and thereby inhibits microtubule-activated ADP release [50]. Different kinesins, different buffers, different temperatures and different acidic carboxy-terminal sequences of tubulin might all tune the stability of the K^{TRAPPED}•ADP state with potentially large effects on the rate of microtubule-activated ADP release from the lead head.

Importantly, microtubule-activated ADP release corresponds to the transition from weak (detachable) to strong (stable) microtubule binding. Figure 1 shows ADP release occurring in two steps: a transition to strong binding, followed by the consequential release of ADP. The weak-to-strong transition thus occurs at a point between two ADP states. Ma and Taylor [5] have

reported that a weak-to-strong transition precedes ADP release. Recent FRET measurements are consistent with (at least) two ADP conformations [51].

This separation of microtubule-activated ADP release into two steps can potentially shed light on a long-standing controversy about whether or not ADP release is rate limiting on the microtubule-activated ATPase [37,46]. The K-loop experiments on the non-processive kinesins Kif1a and Kif1d, as well as the loop-12 experiments on Kif5b [49,50], establish that the strength of the electrostatic attraction between kinesin heads and the microtubule influences the transition rate from weak to strong binding. In at least some situations, the weak-to-strong transition could thus limit the rate of ADP release. Thus, the chemical step of ADP release could be faster and not in itself rate limiting on the Kif5b cycle. The chemical step of ADP release does seem to be rate limiting for Ncd [43,52] and Eg5 [53], two non-processive (Box 1) kinesins [52,54,55].

ADP-driven open-to-closed transition

In Figure 1, the open-to-closed transition can be driven by the binding of Mg•ADP as well as Mg•ATP. Two pieces of evidence support this ADP-driven process: first, several kinesin crystal structures have ADP in the active site but are in the closed (salt-bridged) conformation, albeit not fully closed (i.e. not fully hydrolysis competent [25]). Second, in recent work Naber *et al.* [56] measured a closing of the active site of K•ADP on binding to microtubules.

Closed-to-trapped (strong-to-weak) transition

The closed-to-trapped transition has not been directly measured. Rapid microtubule-activated release of ADP on mixing K•ADP and microtubules ($\sim 80\text{--}300\text{ s}^{-1}$) [36] and rapid dissociation of the kinesin–microtubule complex induced by ATP and ADP ($50\text{--}100\text{ s}^{-1}$) both imply a fast equilibrium at this step. Stabilizing the attached weak K^{TRAPPED}•ADP state by mutagenically increasing its electrostatic attraction to microtubules [50] strengthens the binding of K•ADP to microtubules but slows down microtubule-activated ADP release. In the Kif1a and Kif1c/d kinesins, a weak binding K•ADP state is heavily populated and such heads can undergo 1-dimensional diffusion along microtubules [49,57].

Microtubule binding and release

Both ADP and ATP can trigger the rapid detachment of single kinesin heads from microtubules, consistent with the K^{TRAPPED}•ADP state being the species that detaches. Ma and Taylor [5] estimated rate constants for the unbinding of single human (K379) heads of $40\text{--}50\text{ s}^{-1}$ for ATP-induced unbinding and $65\text{--}90\text{ s}^{-1}$ for ADP-induced unbinding. Jiang and Hackney [58] estimated 46 s^{-1} and 52 s^{-1} , respectively, for a *Drosophila* (DKH357) construct. Gilbert *et al.* [37] obtained 13 s^{-1} for ATP-induced unbinding of *Drosophila* K401, which is predominantly a dimer, but noted a slower phase in their data. Moyer *et al.* [59] measured $21\text{--}22\text{ s}^{-1}$ for the rate of ATP-induced detachment of *Drosophila* K340, but again noted an additional slow process. Crevel *et al.* [2] obtained

Box 1. Processivity

The term 'processivity' is used widely in the literature on molecular motors.

'Mechanical processivity' is the number of steps that the motor molecule takes per productive encounter with the microtubule. Mechanically processive motors or 'porters' (Figure 1a) bind to microtubules and then take multiple steps. Nonprocessive motors or 'rowers' (Figure 1b) bind, undergo a conformational change that costs one molecule of ATP, and then dissociate. Thus, nonprocessive motors need to operate in collaborative teams to maintain contact with the microtubule.

'Kinetic processivity' is the number of ATP molecules that are turned over per motor molecule per encounter with the microtubule. In a processive kinesin, the rate at which the first (intrinsic) ADP is released depends on the microtubule encounter rate and thus on the microtubule concentration, whereas the rate of subsequent ATP turnovers is independent of the microtubule concentration because the motor is already bound to the microtubule. In a walking dimer, the trail head repeatedly detaches and reattaches further along the same microtubule at a rate that depends only on the concentration of ATP and on the local microtubule landscape, and not on the external microtubule concentration. For both processive monomers and processive dimers, if the motor hydrolyses 100 molecules of ATP per encounter, then 99 fewer microtubule encounters than are needed by a nonprocessive motor will be required to turn over 100 molecules of ATP.

'Chemical kinetic processivity' can be recognized when the concentration of microtubules required for half-maximal ADP release at the first turnover is larger than that required for half-maximal ADP release at subsequent turnovers [46]. The number of steps per encounter is given by the equation (maximum cycle rate)/[(microtubule concentration giving half-maximal cycle rate) × (rate constant for ADP release)] [76], provided that one molecule of ATP is used per step. A simpler method is to divide the cycle rate by the detachment rate, although this requires measurement of the detachment rate.

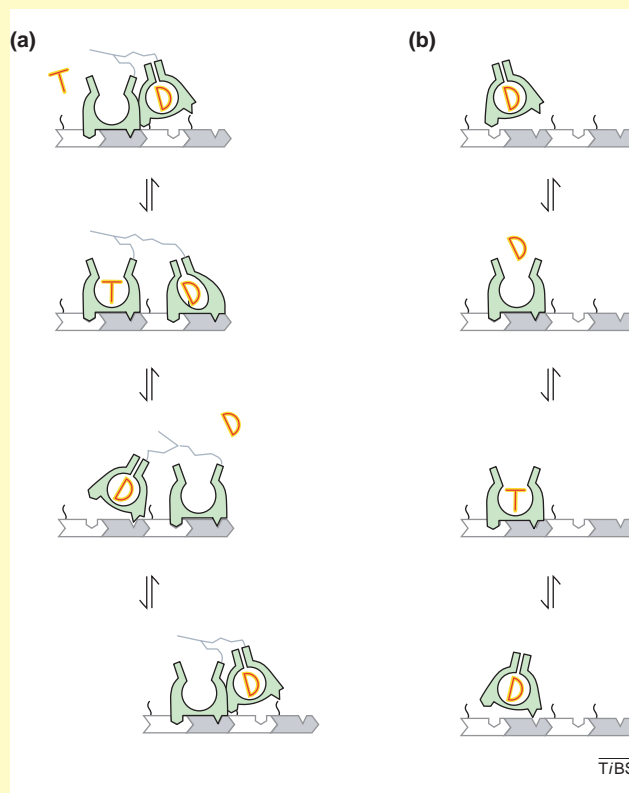


Figure 1. Types of processivity. (a) For a mechanically processive kinesin, ATP (T) turnover is tightly coupled to stepping and kinetic processivity (number of ATP turnovers per molecule encounter) equals mechanical processivity (number of mechanical steps per encounter). (D represents ADP.) (b) Importantly however, some constructs (e.g. monomer constructs with truncated neck linkers [58]) show kinetic processivity but no mechanical processivity.

improved signal to noise and reported 40 s^{-1} for the detachment rate of single heads in ADP and 74 s^{-1} for that in ATP. Single-molecule measurements have also been made using video microscopy [60,61]. In general, video microscopy could underestimate the unbinding rate constant because the unbinding species are large and might tend to rebind before they diffuse out of range of the microtubule.

The detailed conformational mechanism by which kinesin unbinds from microtubules remains to be elucidated. Large-scale conformational rearrangements in response to a switch from ADP to AMPPNP are apparent by microscopy [62] and using proteolytic [31] and FRET [32] probes. Truncation of the neck linker seems to inhibit ATP-induced dissociation of kinesin monomers [15], suggesting that a neck-linker transition might be important in triggering unbinding.

Strain dependence and the kinetic coordination of molecular walking

For Kif5b, a tight 1:1 coupling of physical steps to ATP consumption occurs at various ATP concentrations, velocities and loads [12,13,63,64]. It follows that the chemical kinetic actions of the two heads are linked.

Recent evidence has established that the two heads do indeed step alternately [14,65], but other aspects of coordination remain controversial. Figure 2 tries to summarize the various schemes as a series of proposed control points (gates) in the stepping mechanism, whereby one head governs the actions of the other.

The first such gate is that discovered by Hackney [41] and confirmed by others [10,44,45] in which an ATP-dependent conformational change in the trail head works as a trigger for microtubule-activated release of ADP from the lead head. Before ATP binding, the trail head is in rigor and the lead head is tethered to prevent it from undergoing microtubule-activated ADP release. ATP binding to the trail head releases this restriction, with an associated shift in neck-linker dynamics [66,67] (Box 2) that enables the lead head to undergo microtubule-activated release of ADP. Lengthening the neck linker by inserting a flexible sequence substantially abolishes the dependence of lead-head ADP release on ATP binding to the trail head [68].

Binding of other nucleotides to the trail head also works to release ADP from the lead head, but the rate is slower (ATP- γ S > AMPPNP > ADP). If ADP binds to the trail head, the ADP-induced detachment of the trail head competes with the ADP-induced release of ADP from the lead head [36]. For *Neurospora* kinesin, which is also

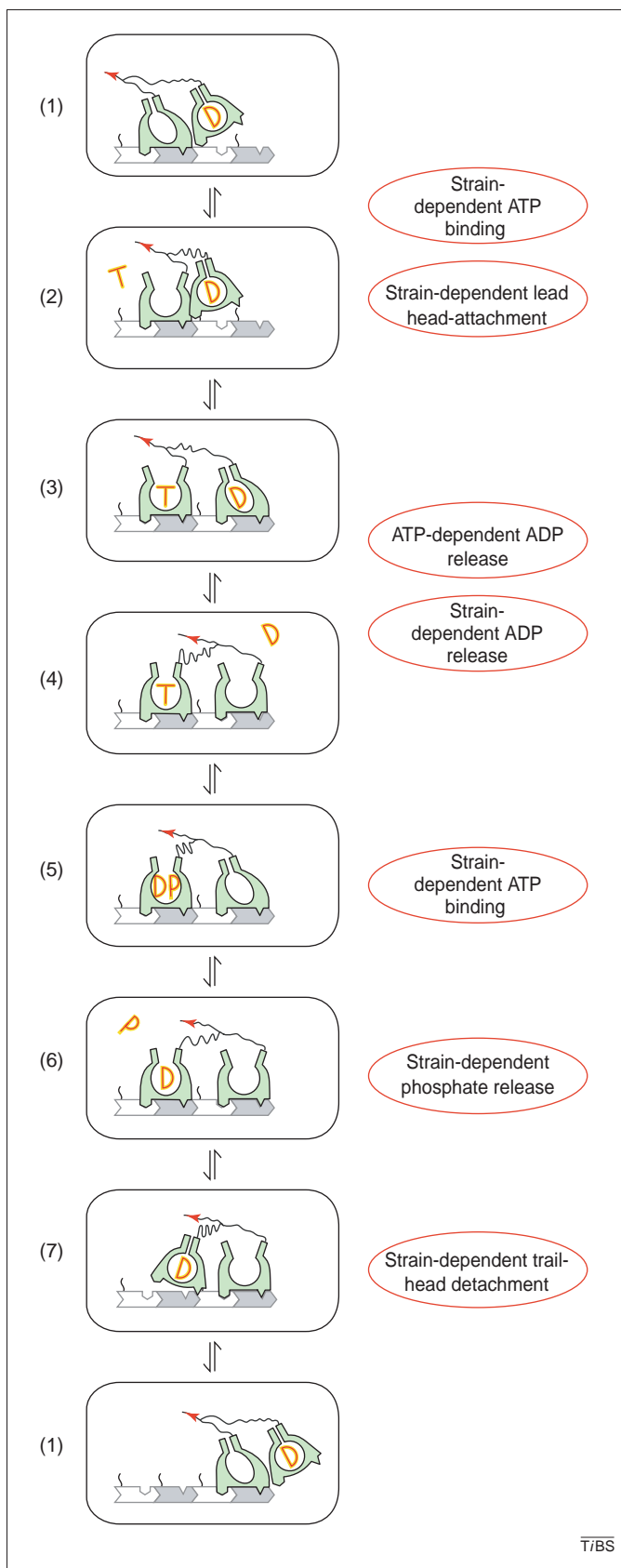


Figure 2. Candidate control points for two-headed walking. This sequence of events attempts to summarize the various candidate control processes, in which the rates of some chemical steps are dependent on strain. Because kinesin can step against an applied load of 6–8 pN, the heads are shown as being strained by an external force (red arrows). The transition 1–2–3 represents ATP-dependent exit from the tethered intermediate (state 1). Initially, ATP (T) binding is inhibited by retroactive strain. After ATP binds to the trail head, ADP (D) release from the

processive, AMPPNP-induced ADP release is 15 times slower than ATP-induced ADP release, suggesting that a conformational change associated with hydrolysis on the trail head (perhaps the open-to-closed transition) is required [45]. Note, however, that some salt-bridge mutants that abrogate hydrolysis retain the ability for nucleotide binding by the trail head to gate attachment of the lead head [26,28].

There is clear evidence for a subsequent mechanochemical gate at the commitment step of ATP binding. Work by Klumpp *et al.* [38] on mutant dimers that are blocked in detachment indicates that ATP binding to the lead head of the mutant dimer is prevented when the trail head cannot detach. Optical trapping experiments also show a decrease in stepping rate (equivalent to the ATP binding rate) with increasing retroactive load [69]. Higuchi and colleagues [64] have reported that back steps also obey this relationship. Schnitzer *et al.* [70] argue for a load-dependent isomerization (conformational change) of a $K \bullet ATP$ state. This strain dependence will inhibit ATP binding to the lead head until detachment of the trail head has occurred, thereby avoiding the potential risk of futile cycling on the lead head at high concentrations of ATP [28,71].

Further possible gates might operate at phosphate release, ADP release and/or trail-head detachment. Schief *et al.* [40] suggest that the trail head must detach before it can release phosphate. The experiment of Klumpp *et al.* [38] contradicts this for a mutant that is blocked in detachment. One possibility that remains is that phosphate release is coupled to a small work stroke, implying that phosphate release and detachment will slow down under load; however, there is no evidence for this at present.

In the scheme in Figure 2, a gate corresponding to ADP release controls the transfer of load from the trail head to the lead head and thus the 8-nm step. Uemura and Ishiwata [72] have reported that kinesin's affinity for ADP is dependent on strain. The model in Figure 2 accordingly couples ADP release to a relative movement of the load and track.

With regard to detachment of the trail head, Schief and Howard [29] measured the rate of ATP-induced detachment of a one-headed kinesin dimer to be less than 1 s^{-1} , which would therefore require a processivity scheme with substantial acceleration of trail-head detachment by lead-head attachment. Hackney [36] also argues for the acceleration of trail-head detachment by lead-head attachment, albeit to a lesser degree, because the ATP-induced unbinding of monomers is too slow to account for the stepping rate of dimers. Recent measurements [2] on the detachment of $K^{\text{TRAPPED}} \bullet ADP$ trail heads of dimers put this acceleration at only around twofold faster than the spontaneous rate. In Figure 2, phosphate release from the trail head accordingly occurs before the trail head detaches, and trail-head

lead head is allowed, but it is also inhibited by retroactive strain (state 3). The 8-nm step happens as ADP release is completed and the load is stably transferred from the trail head to the lead head (transition 3–4). ATP binding to the lead head is then inhibited by retroactive strain, enabling hydrolysis and phosphate release on the trail head to be completed (transition 4–5–6). Trail-head detachment in the $K^{\text{TRAPPED}} \bullet ADP$ state (transition 6–7) is accelerated slightly by a pull from the lead head and regenerates the tethered intermediate on the next tubulin heterodimer along, thereby completing the cycle.

Box 2. Neck-linker dynamics

Crystal structures of kinesin monomers contain a neck-linker domain of ~15 residues that either can be mobile (and hence invisible in the crystal structure) or can dock against the $\beta 1$ sheet of the head. Rice and colleagues [26] have reported that, in the absence of microtubules, the docked–free equilibrium for single heads is only slightly biased by different nucleotides, but that microtubule binding produces a stronger coupling between the neck-linker conformation and the contents of the active site, with $K \bullet \text{AMPPNP}$ having predominantly a docked neck linker and the apo-kinesin and $K \bullet \text{ADP}$ states having predominantly an undocked neck linker.

On the basis of these findings, which relate to monomers, Rice *et al.* [26] proposed an initial model in which neck-linker docking was the fundamental driver for motility of walking dimers. A more recent analysis has shown that the energy involved in docking and undocking is lower (~1 kT) than that of the full kinesin power stroke (~10 kT) [66]. A modified model proposes two roles for neck-linker docking in the walking mechanism [66]. First, ATP-dependent neck-linker docking is proposed to position the tethered head close to its next site along the microtubule, thereby biasing a diffusional search made by the tethered head for its next site (Figure 1, top and middle). This role is consistent with cryo-electron microscopy images showing that tethered heads point away from their next sites in the ADP and rigor states, but towards their next sites in the AMPPNP state [75]. Second, undocking and melting of the neck linker on the trail head are proposed to follow ATP hydrolysis and to create an entropic spring that links the two heads, such that the trail head is pulled off (Figure 1, bottom). Note also that neck linkers powerfully enhance the detachment of single heads [15], necessarily by a mechanism other than the proposed entropic spring.

Mutagenesis [77] and crosslinking [78] studies are consistent with ATP-dependent neck-linker docking being a device for enabling and enhancing processivity. It might function by contributing directional bias to the diffusional component of a step that comprises both diffusional and power-stroke components.

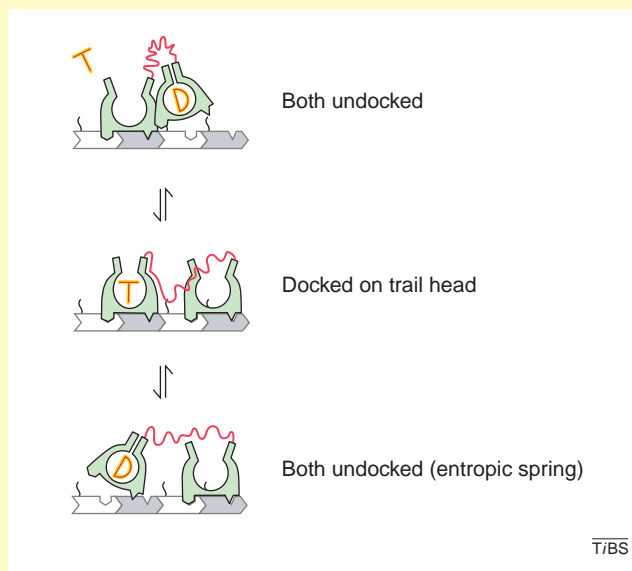


Figure 1. Dynamics of the kinesin neck linker. ATP (T) binding docks the neck linker (red), thereby, posing the leading (tethered) head close to its next site on the microtubule. In ADP (D) and empty states, the neck linker is undocked and melted.

detachment as the $K^{\text{TRAPPED}} \bullet \text{ADP}$ state is accelerated by plus-end-directed strain from the newly attached lead head.

Other kinesins

Owing to limitations of space, only passing reference can be made to kinesins other than the canonical Kif5b. The

available evidence indicates that many, if not most, kinesins are nonprocessive (i.e. they touch down to the microtubule, undergo a power stroke and then detach) and therefore need to operate in teams to maintain contact with the microtubule. All kinesins, including the minus-end-directed kinesins [43,52] and even the Kin I kinesins that actively depolymerize microtubules [73], seem to share broadly the same kinetic mechanism.

Future prospects

Kinesin is a mechanoenzyme: the whole purpose of its cycle of microtubule-activated ATP turnover is to produce strain and to use the associated strain energy to drive motions. Because chemically driven conformational changes produce strain [74], applied force can alter conformation and with it chemical kinetic rate constants. Determining the strain dependence of the active-site chemistry will be key to understanding both the force-generating mechanism of individual kinesin heads and the coordination of linked heads.

In principle, all transitions between attached states in the cycle might correspond to mechanical displacements and hence might be strain dependent. Although it is possible that a major displacement (a working stroke) can be mapped to a single chemical step, structural rearrangements occur at several points in the kinetic cycle [75], and each could potentially produce a productive displacement. With regard to this, all kinesins contain a common catalytic core, but the manner in which conformational changes in the core are harnessed can differ markedly between subfamilies, for example, between plus-end-directed and minus-end-directed kinesins. It will be important to understand this in more detail.

A challenge for the future will be to unpack the mechanisms of teamwork in teams of more than two heads. For the processive Kif5b, however, perhaps the salient problem at present is to measure the mechanical displacement and strain dependence of each step in the chemical kinetic cycle of ATP turnover.

Acknowledgements

The author thanks the four expert reviewers of this article for their careful and constructive criticism.

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