Coordination between Motor Domains in Processive Kinesins*

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Elena P. Sablin and Robert J. Fletterick

From the Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

Among cellular functions that kinesins perform, their ability to walk along the microtubules transporting specific cargoes is the most fascinating (1, 2). The complexity of this phenomenon and tremendous scientific efforts put toward dissecting the mechanisms underlying it have led to conflicting models explaining how kinesins might travel long distances without disassociating from their biological track (e.g. move “processively” (3)).

Although these models differ, for conventional kinesin dimer the favored one predicts that the two kinesin heads bind alternately to the track, taking 80-Å steps along the microtubule while hydroylizing one ATP molecule per step (4–6). This “hand-over-hand” model (7, 8) also predicts that the two kinesin heads remain enzymatically “out of phase” (e.g. at different stages of the ATP hydrolysis cycle), ensuring that, at any given tug, at least one of the two motor domains remains strongly attached to the track.

Many technologically marvelous papers (9–18) illustrate the importance of coordination between the motor domains of kinesin during its processive movement. We review the phenomenon of kinesin processivity from a complementary perspective by considering specific structural features of the motor domains that underlie their coordination.

Structural Features That Transform a Protein into a Processive Motor

All kinesins share the conserved catalytic core (residues Asn̈–Ala232 in human kinesin (19)), which consists of a central β-sheet sandwiched between six α-helices (Fig. 1A, cream) and a topologically conserved smaller lobe (called the β-domain here) (Fig. 1A, peach) with three additional β-strands. The core has both the nucleotide- (Fig. 1A, green) and microtubule-binding sites (the major site, loop L12 and α4, is indicated in Fig. 1B) and with the microtubule executes nucleotide hydrolysis cycle powering kinesin motility (20, 21). Depending on its nucleotide state (γ-phosphate either present (ATP/ADP-P) or absent (ADP/no nucleotide)), the catalytic core “switches” between different conformations (6, 22), controlling affinity of kinesin for the microtubule and the positions of the force-generating mechanical element of the motor, the neck region (effectively a transmission (20, 21, 23–25)). In conventional kinesin, the neck (19–21, 26) is positioned C-terminally to the core and consists of the neck linker (residues Lys323–Thr336 in human kinesin) (Fig. 1A, blue) and the following neck coiled coil (Fig. 1A, gray), which maintains the dimeric state of the motor.

Fig. 1, B and C, shows how the major switching region, “switch II cluster” (22, 27), rising above the central β-sheet in the presence of γ-phosphate (blue) and collapsing back in its absence (red) controls the conformations of the neck by, respectively, either facilitating docking of the neck linker and its base, Ile325, along the core or sterically precluding this docked position. In the microtubule-bound motor, the power stroke is facilitated by repositioning the neck linker from its nucleotide-free conformation into the docked, ATP-like state, an assembly that propels the partner motor subunit toward the plus end of the microtubule (20, 25).

Because of their proximity and inseparable functional roles, the catalytic core and the adjacent neck have been termed the motor domain (19, 20). In the conventional kinesin, the nucleotide-induced conformational transitions in one motor domain are amplified into 80-Å step movements by the partner motor domain, which is connected through the neck coiled coil and the following stalk domain (6, 20, 25).

The alternating force-generating repositioning of the neck in kinesin motor domains is achieved when one of the two domains is firmly attached to the track (between the strongly bound nucleotide-free and ATP states (6, 25, 28)) and, therefore, can use the produced force to displace the partner motor domain forward. The coordinated control over the affinity of kinesin for the track is achieved by the nucleotide-dependent restructuring of the microtubule-binding sites of the motor, which are positioned either directly within the nucleotide-responsive switch II cluster (L12 and α4, Fig. 1B) or close to it (27).

In addition to the coordination between the affinity of the motor for the track and its power-generating movements, processivity of conventional kinesin requires special properties of its neck. Stability of the neck coiled coil, which does not unwind during movement of kinesin (29), keeps the two motor domains in register. At the same time, flexibility of the neck linker (25) allows the motor domains to propel over each other, optimizing their positions while searching for the new binding site. The ability of the neck linker to extend allows bridging the 80-Å distance between neighboring binding sites on the track (30). In non-processive kinesins, the necks are evolved for performing different functions (for optimizing the plus end-directed power stroke in the mitotic spindle kinesin Eg5 (31) or for producing the oppositely directed force in the minus end-directed motor Ncd (32–36)).

Cooperativity between Motor Domains Drives Processive Movement of Kinesin

Structural (30) and biophysical (15, 18, 37) data show that at a critical point of its movement conventional kinesin adopts a configuration on the microtubule with both motor domains bound to the track. In this “bridged” state (Fig. 2A), the trailing motor is firmly attached to the microtubule, has ATP/ADP-P, in its nucleotide site, and adopts the ATP-like conformation with the neck linker docked alongside the core and pointing (from N to C termini) toward the plus end of the microtubule (30) (Fig. 2A, blue). This configuration allows the leading motor to attach to the next site on the protofilament, releasing ADP and adopting the nucleotide-free conformation with the neck linker pointing in the opposite direction (30) (Fig. 2A, red). Kinetic data show that the
leading motor cannot bind ATP until the trailing motor hydrolyzes ATP and releases one of the hydrolysis products, P\textsubscript{i} (18). This release weakens attachment of the motor core to the microtubule, and the trailing motor detaches from the track, losing constraints placed onto the leading motor and allowing it to bind ATP and enter its nucleotide hydrolysis cycle (18).

The inability of the leading motor to enter a new hydrolysis cycle until the trailing motor reaches the end of the previous cycle and is prepared to bind to the next site on the track is critical for processive movement (9, 13, 15, 18). This kinetic coordination guarantees that the leading motor would not prematurely enter and finish its ATP hydrolysis together with the trailing motor domain detaching from the microtubule and causing the motor dimer to lose its grip with the track.

**Interactions of Kinesin with the Microtubule Track**

Although kinetic cooperativity between the kinesin motor domains is proven and its functional importance is well understood, the structural mechanism underlying this phenomenon is fuzzy.

With eight structures of plus end-directed kinesin motor domains determined and some observed in different conformational states (22, 31, 38–42), the character and the amplitude of conformational motions that kinesin would undergo during its movement can be analyzed. By coupling the crystal structures with electron microscopy images at improved resolution (22, 43), the kinesin motor domain can be positioned on its track in different nucleotide states.

We analyzed conventional kinesin in its bridged state (Fig. 2B), looking for determinants that would explain the cooperativity between the two motor domains. For this analysis, the trailing motor with the docked neck linker (Fig. 2B, blue) was modeled from the structure of kinesin in the ATP-like conformation (42). Its position on the track was taken to be equivalent to that determined for KIF1A in the ATP-like state (22). The position of the leading core (Fig. 2B, gray) was adopted from the alternative orientation observed for KIF1A in the ADP/no nucleotide state (22). The leading core in

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**Fig. 1. Structural organization of conventional kinesin.** A, the catalytic core, \(\beta\)-domain, and bound nucleotide of conventional kinesin (3kin) are, respectively, in *cream*, *peach*, and *green*. The neck linker and coiled coil are *blue* and *gray*, respectively. B, mechanism of conformational switching is exemplified using superimposed atomic models for kinesin in ADP (1bg2) and ATP-like (1mkj) states. Switch II cluster and neck linker are *blue* for ATP-like and *red* for ADP states. C, mechanism underlying the nucleotide-dependent conformational transitions of the kinesin neck.

**Fig. 2. Bridged state of conventional kinesin.** A, schematic representation of the bridged state. The catalytic core is *cream*, the \(\beta\)-domain is *yellow/red*, and the oppositely directed neck linkers of the trailing and leading motors are *blue* and *red* arrows, respectively. The neck coiled coil is drawn as a *braid*. The trailing motor (with either ATP (T) or ADP, (DP)) and the leading core (nucleotide-free) are strongly attached (indicated by *anchors*) to the microtubule. B, an atomic model for the bridged state of conventional kinesin. The coloring scheme is consistent with Fig. 1A and A above. The countermovement of the leading core is indicated by arrows. Polypeptide chains for \(\alpha\) and \(\beta\)-tubulin (1tub) are in *dark* and *light gray*. 

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**Minireview: Coordination in Kinesin Motors**

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this dimer configuration is translated toward the minus end of
the microtubule (by −3 Å) and rotated clockwise (by −10 degrees, Fig. 2B) relative to the position of the trailing core bound
to the equivalent tubulin dimer. The mechanism underlying
this changed orientation has been explained by the compensa-
tory countermovement of the motor relative to its switch II
cluster, which changes its position relative to the core between
the nucleotide-free and ATP-bound states (Fig. 1B) (22, 27). In
the microtubule-bound motor, its movement relative to the
microtubule might be restrained by anchoring interactions
with the track in both strongly attached states (22, 27).

For both dimers, we modeled the neck linker of the leading
motor domain in the orientation opposite to that of the trailing
motor (Fig. 2B, red). In placing the leading linker, we used two
constraints. The first was the position of the N terminus of
the leading neck coiled coil that marks the end point of the leading
neck linker. The second was the observation that helix α6
preceeding the neck linker can unwind only at its C terminus
(two residues C-terminal to Arg121). In all kinesin structures,
the rest of the helix remains stable because of packing inter-
actions with the core. Thus, for both kinesin dimers the start
and the end of the leading neck linker have been defined.

In both kinesin configurations, the leading neck linker must
adopt an extended conformation to bridge the preceding helix
α6 and the following neck coiled coil. In both dimers, the
leading linker runs alongside the β-domain of the leading core
(Fig. 2B, peach), making contacts with this structural element
unavoidable. However, in the first dimer (not shown) because of
the orientation of the core and the position of the small lobe, the
neck linker abrades the β-domain. Attempts to avoid this clash
resulted in the linker being stretched beyond the geometry
suitable for the polypeptide chain, making this configuration of
the double head-bound kinesin unlikely.

Reorientation of the leading motor in the second dimer by the
countermovement of the core (Fig. 2B) positions the β-domain compatibly with the conformation of the leading neck linker.
Remaining extended, the linker runs alongside the β-domain in
this bridged kinesin dimer, making side chain contacts with one of the β-strands of the small lobe, β1c. Notably, the neck
linker of kinesin motor Eg5 has been observed in a similar
conformation. Furthermore, this conformation correlated with
the ADP/no nucleotide-like state of the motor (31).

A Structural Model for Coordination between Kinesin Motor Domains

A structural model for kinetic and mechanical cooperativity
between the motor domains of conventional kinesin is proposed
in Fig. 3. This model explains why the leading motor is unable
to bind a new molecule of ATP (Fig. 3B) until the trailing motor
hydrolyzes the previous one and, having ADP in its nucleotide
site, becomes either weakly bound or detached from the micro-
tubule (Fig. 3C). Only after detachment of the trailing motor
(Fig. 3C) the leading neck linker relaxes and allows the leading
core to reorient itself on the microtubule. This reorientation likely brings the nucleotide-binding elements of the motor
(switch I and switch II regions (27)) into the proper position
relative to each other and to the stabilizing microtubule sur-
face, facilitating new nucleotide binding. The resulting reori-
entation of the leading neck linker into the alternative, ATP-
like position (Fig. 3D) further stabilizes the ATP-bound conformation of the motor, allowing nucleotide hydrolysis. In a
sense, the extended leading neck linker in the constrained,
bridged state of kinesin (Fig. 3B) acts as a structural sensor
that coordinates work of the enzymatic active sites in two kinesin cores keeping them “out of phase.”

We favor the suggested model because it explains the ob-
served cooperativity between kinesin motor domains and is

![Figure 3](https://example.com/figure3.png)

**Fig. 3. Structural model for cooperativity between motor do-
 mains of conventional kinesin.** Symbols and their coloring scheme are consistent with Fig. 2A. A relaxed state of the neck linker in the
ADP (D) state is indicated by the contoured red arrow.

built using crystal structures (38, 42) and experimentally ob-
served orientations of the motor on the microtubule (22). Fur-
thermore, for both the leading and trailing motors, these con-
formations and orientations are consistent with the general
switching mechanism proposed for kinesins (6, 22, 27). The
model also explains the existence of the distinct and topologi-
cally conserved feature of kinesin, the β-domain, whose func-
tional role remained mysterious. In its newly proposed role for
conventional kinesin, β-domain would function similar to the
extended β-strands of the central core, stabilizing and pointing
the neck linker either toward plus or minus end directions in
the ATP/ADP-P, and nucleotide-free states of the motor, respec-
tively. In non-processive plus end-directed kinesins, the β-
domain would serve a similar function, stabilizing the ADP/nu-
cleotide-free conformation of the neck linker and defining the
amplitude of the power stroke of the motor (31). Recent struc-
tural studies (35, 36) of the minus end-directed motor Ncd
suggested that the amplitude of the oppositely directed power
stroke might be defined by interdomain interactions modulated
by the β-domain.

Packing interactions of the conventional neck linker with the
β-domain in the nucleotide-free state are less extensive com-
pared with interactions of the linker with the core in the
ATP-like state. For this reason, the docked conformation of the
linker in the nucleotide-free motor may be less stable. In the
bridged state of kinesin, this conformation is enforced by the
trailing motor tugging the leading neck in the minus end di-
rection. Consistent with this idea, the neck linker of the nucle-
otide-free microtubule-bound monomeric kinesin is flexible and
adopts multiple conformations (25, 30). However, some of these
conformations seen both in nucleotide-free and ADP-bound
motor (25) are consistent with the neck linker being oriented
backwards and docked along the β-domain.

The function of the β-domain in conventional kinesin likely
extends beyond its role in supporting the nucleotide-free con-
formation of the leading neck. Upon binding ATP, the counter-
movement of the leading core and resulting repositioning of the β-domain would dislodge the neck linker from the small lobe (similar to dislodging the neck from its docked position on the central core by the collapsing switch II cluster upon nucleotide hydrolysis (6, 22, 27)). Furthermore, the ATP-induced counter movement of the core would bring the N-terminal base of the neck (conserved small hydrophobic residue, Ile325 in human kinesin, Fig. 1C) closer to the hydrophobic pocket underneath the switch II cluster (6, 22, 27). In the extended conformation of the linker docked on the β-domain, Ile325 is placed ~10 Å from its position in the ATP-like state (Fig. 1C) and, therefore, is unlikely to move into the pocket because of hydrophobic interactions. The assisted repositioning of the base of the neck would facilitate docking of Ile325, triggering docking of the entire neck linker along the core in the ATP state (25). Because the transition between two alternative docked conformations of the neck linker would not rely on brownian motion only but would be forced by the countermovement of the core, placement of the detached trailing motor toward the new site on the track could be achieved more efficiently. Importantly, the countermovement of the leading motor upon binding ATP would also prevent the trailing motor from rebinding to the same site on the microtubule.

Electron microscopy and crystallographic data (25, 31) suggest that the backward orientation of the neck linker could be achieved not only in the nucleotide-free but also in the ADP state of kinesin. These observations support the proposed model (Fig. 3), which predicts that even transient docking of the neck linker along the β-domain in the unattached ADP-bound motor domain (Fig. 3A) would make repositioning of this domain toward the next binding site more energetically favorable. The free-energy gain would come from favorable enthalpy changes balancing out unfavorable entropy changes associated with straightening of the leading neck linker (Fig. 3, A and B) during forward stepping (46).

Future Challenges

High-resolution structures of the kinesin motor domain complexed with tubulin polymer at different stages of the mechanochemical cycle of the motor would, no doubt, clarify many of the questions raised in this review. So far, moderate resolution of the available electron microscopy images of the kinesin bound to its track prevent us from dissecting the exact structural mechanisms underlying the processive movement of the motor.

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