Minireview

A look into kinesin's powerhouse

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Abstract Kinesins are microtubule-dependent motors that serve a multitude of cellular purposes. The conserved motor domain provides the energy required for these processes. Shortly after the solution of the first kinesin motor domain crystal structures the similarity to myosin and G-proteins was noted. By analogy, it was suspected that regions flanking the γ-phosphate group of the nucleotide (in particular the so-called switch I and II regions) play important roles in the catalytic mechanism and the communication between the nucleotide cleft and the microtubule binding site. Since then, mutational analyses have supported this notion. Moreover, additional high-resolution structures have demonstrated that the switch regions can assume variable conformations. In one case, a comparison of an ADP state and an ATP-like state indicates a crucial involvement of the helix flanking switch II in modulating microtubule affinity. High-resolution structures of a kinesin-related protein mutated in the switch regions confirm the correlation between structural features in the switch vicinity and coupling of microtubule binding and nucleotide state.

Key words: Kinesin; Motor protein; P-loop; Crystal structure; ATPase

1. Physiological functions of kinesin-related proteins (KRPs)

Kinesin is a protein capable of transporting cellular cargo along microtubules using the chemical energy derived from the the hydrolysis of ATP. Originally, it was identified in motility assays of squid axoplasmic extracts, where it presumably drives vesicle movement [1]. This type of kinesin has now been termed conventional kinesin, and has been found in different animal and fungal species [2]. Although the cargo(es) of conventional kinesins are as yet poorly defined, they most likely are responsible for the transport a wide variety of membranous structures within the cell. In contrast to the other class of microtubule-dependent motors, the dyneins, or the actin-dependent motor myosin, kinesin's motor domain is much smaller and fully functional without additional polypeptide chains. It has also turned out that conventional kinesins are able to move large distances along the filament as single molecules without detaching, a property referred to as 'processivity', implying a highly coordinated mechanism of motility [3]. Since the movement of conventional kinesin is strictly unidirectional towards the plus-end of microtubules, it appears that it has evolved as a motor for anterograde long-distance transport with velocities of up to 3 μm/s [2].

Soon after conventional kinesins, other proteins were discovered that share a high degree of amino acid identity in the motor domain but are completely unrelated in their non-motor domains. Today, at least nine subfamilies of such KRPs are known. Some of them are also vesicle motors, but many are implicated in mitotic and meiotic events. The yeast Kar3p motor, for example, was discovered as a mutant impaired in karyogamy and is now thought to play a role in nuclear migration [4]. A knockout mutant, however, also shows defects in mitosis where it acts in combination with two other KRPs, Kip1 and BimC [5]. These three motors appear to build up a system that balances the forces occuring during mitosis, and may therefore not be used primarily to drive motility but to generate force.

A third type of cellular function has been suggested for members of the kinesin I (MCAK, KIF2) family of motors [6]. These kinesins are able to depolymerize microtubules in an ATP-dependent fashion but do not generate movement. Depolymerization can take place at either end of the microtubule by a mechanism that is not yet understood. The ATP-dependence suggests that these kinesins are adapted to influence microtubule stability.

Functions other than the three outlined here may be identified in the future since not all known kinesins have been functionally characterized yet. However, since all kinesins share homologous motor domains, their key mechanism of action is probably conserved. As the core motor domain functions as a microtubule-activated ATPase, the common feature is the ability to couple nucleotide and microtubule binding states, and to utilize the conformational changes in the motor core for dedicated purposes. The modular concept seen in the domain organization may reflect a functional necessity, and different domains may be required to convert the energy from hydrolysis into either force, processive movement, modulation of microtubule dynamics, or some other, as yet unknown, output.

2. Structural basis of kinesin function

During the last 5 years, several kinesin crystal structures have been solved ([7–16], Table 1). The structures do not comprise full-length proteins but only core motor domains with adjacent regions in some cases (up to approximately 90

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Table 1
Crystal structures of KRPs available from the Protein Data Bank (www.rcsb.org)

Kinesin	PDB access	Nucleotide state	Oligomerization	Comments
Human conventional kinesin	1BG2	Mg–ADP	monomeric	Neck-linker disordered [7]
Rat conventional kinesin	2KIN	ADP	monomeric	Neck-linker and partial neck ordered [9]
	3KIN	ADP	dimeric	Neck coiled-coil ordered [10]
Drosophila melanogaster ncd	2NCD	ADP	dimeric	[8,11]
	1CZ7	Mg–ADP	dimeric	[12]
Saccharomyces cerevisiae Kar3p	3KAR	Mg–ADP	monomeric	[13]
	1F9T	Mg–ADP	monomeric	[14]
	1F9U	Mg–ADP	monomeric	Point mutant in helix α4 [14]
	1F9V	Mg–ADP	monomeric	Point mutant in switch I [14]
	1F9W	Mg–ADP	monomeric	Point mutant in switch II [14]
Mouse Kif1A	1I5S	Mg–ADP	monomeric	[15]
	1I6I	Mg–AMP-PCP	monomeric	The only non-ADP structure [15]
Human Eg5	1II6	Mg–ADP	monomeric	Novel conformation of neck-linker [16]

extra amino acids). None of the high-resolution structures reflect the microtubule-bound state. Most crystal structures are of monomers with bound ADP, but for rat, conventional kinesin and *Drosophila* ncd dimeric structures are available, while for the Kif1A kinesin, a conformation containing a non-hydrolyzable ATP analogue has been solved. What do these structures teach us?

It has been noticed early that the catalytic cores of the kinesin motor as well as of myosin and G-proteins share common motifs in the vicinity of their respective nucleotides [17]. In in all three classes of proteins, nucleotide binding is accomplished via a P-loop motif, while the γ-phosphate groups are flanked by highly conserved regions termed switch

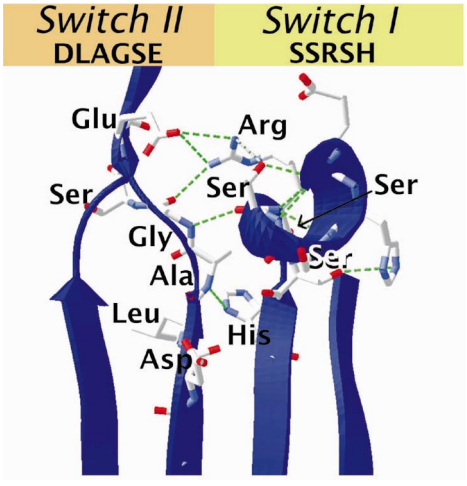

Fig. 1. Switch I and II regions in the catalytic kinesin motor domain. The figure shows a slab view from the nucleotide towards the switch regions. The left part of the picture shows switch II (sequence DLAGSE), and the right shows switch I (sequence SSRSH). The (invisible) γ-phosphate of ATP would be located in front of the switch II glycine. In this monomeric rat kinesin structure, several interswitch hydrogen bonds are visible. Special attention has been paid to the salt bridge between switch I Arg and switch II Glu because the large side chains may allow conformational mobility. The central switch II glycine is conserved among kinesins, myosins and G-proteins where it is known to 'sense' whether the γ-phosphate group is present (GTP state) or not (GDP state, [17]). Therefore, it is likely that in kinesin, too, this glycine is crucial for transmitting the nucleotide state to the microtubule binding region, thus modulating the affinity for the filament. Although not every hydrogen bond is clearly visible in each crystal structure, the key elements are present, and differences may be explained by experimental conditions or minor functional modifications.

I and switch II. In kinesin, the amino acid sequences in these switch regions are highly conserved. Switch I is formed by a stretch S-S-R-S-H, switch II by D-L-A-G-S-E. Both regions are linked via a complex network of hydrogen bonds (Fig. 1). By analogy to myosin and G-proteins, a rearrangement of this system may trigger conformational changes that modulate microtubule affinity as well as secondary changes that lead to the generation of motility or force. In this cascade of events, a crucial role is played by a helix in the vicinity of switch II, the so-called switch II helix, which may shift, twist or elongate, depending on the nucleotide state. Since kinesin's major microtubule interaction site is thought to be located near the end of the switch II helix [18,19], its central function in kinesin mechanochemistry is further emphasized.

The hypothesis that the switch regions transmit the state of the nucleotide to the microtubule binding site has been substantiated by recent crystallographic studies [14,15]. The crystal structure of Kif1A has provided the first insight into possible links between the nucleotide state and conformational changes [15]. Thus, whereas the ATP-like structure obtained in the presence of the non-hydrolyzable ATP analogue β,γ-methyleneadenosine 5-triphosphate (AMP-PCP) exhibits a salt bridge between switch I Arg and switch II Glu, the ADP conformation does not, because the Arg moves outwards from the nucleotide binding cleft (Fig. 2). This movement is caused by a transition of switch II from a hairpin to a helical structure. The switch II Glu behaves oppositely and moves a large distance into the nucleotide cleft. The displacement of this residue has a huge impact on the switch II helix: it rotates by approximately 20° with respect to the rest of the molecule. According to the authors, this twist may influence the microtubule affinity, with the AMP-PCP conformation displaying a tighter binding.

There are a couple of critical issues in this interpretation. When the Kif1A structures are compared with conventional kinesin from rat, the similarity of the Kif1A AMP-PCP and rat kinesin ADP conformations becomes obvious (Fig. 3). The position of the switch II helix in the Kif1A ADP conformation, however, is quite different. But, since the human conventional kinesin ADP conformation resembles closer the Kif1A ADP state, there seems to be no strictly coupled, universal ADP conformation. The only valid conclusion may be that the switch II helix is mobile. This has been predicted theoretically and may be relevant for the understanding of communication between the nucleotide binding cleft and the microtubule binding domain [20]. Remarkably, the corresponding

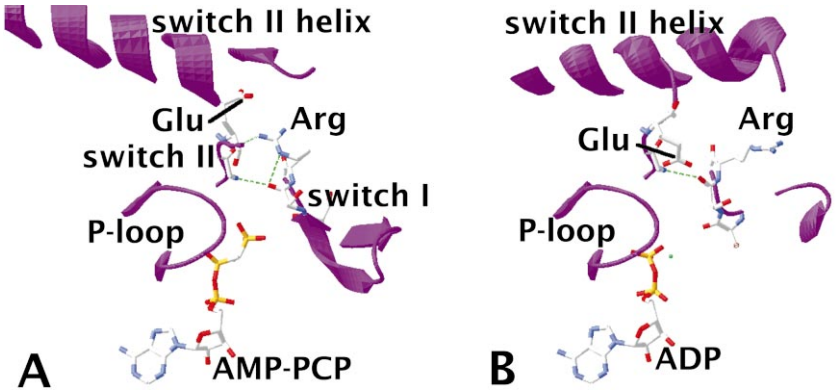

Fig. 2. Nucleotide-dependent rearrangements in the switch I and II regions of Kif1A [15]. Panel A shows a slab view of the nucleotide environment in the AMP-PCP state, panel B, the ADP state. The structures are aligned on their P-loops. Large movements of the switch I R and the switch II E are obvious, leading to conformational changes of switch I and a tilt of the switch II helix.

helix in myosin ('relay helix') also shows a nucleotide-dependent movement [21]. However, the lack of a high-resolution filament-bound structure prevents an unambiguous correlation between a particular helix conformation and the microtubule binding state.

The relation of the switch II helix structure and the microtubule binding state may be even more complex than described so far. In other KRPs, certain modifications in the switch II helix are observed. Thus, Kar3p and Eg5 display a longer helix that extends N-terminally into the vicinity of the switch I/II regions. The conventional kinesin NcKin from *Neurospora crassa* possesses an additional short helix formed by residues in the switch II vicinity that connects to the N-terminal end of the switch II helix via four non-helical residues (Y.H. Song and E. Mandelkow, personal communication). These observations hint at the importance of regions linking the nucleotide binding site and the switch II helix for the velocity of kinesin. Kar3 and Eg5 are slow motors, whereas NcKin is the fastest kinesin known.

An exciting study offers additional insights into the importance of the switch I/II regions and the switch II helix [14]. Kar3p was used as a model motor and crystallized in four different versions: in addition to the wild-type motor domain, three point mutants were used: one in switch I, the second in switch II, and a third in the switch II helix (Table 2). In switch I and II, the two presumptive salt bridge residues (switch I Arg and switch II Glu) were replaced by alanines. The R→A mutation lead to a disorder in switch I but caused the loop that links switch II and the switch II helix to be ordered. The mutation has a dramatic phenotype and is essentially unable to bind to microtubules. In contrast, the E→A replacement mutant was impaired in its release from microtubules. Here, switch I as well as switch II was disordered. These data support the mutual dependence of switch I/II conformation and

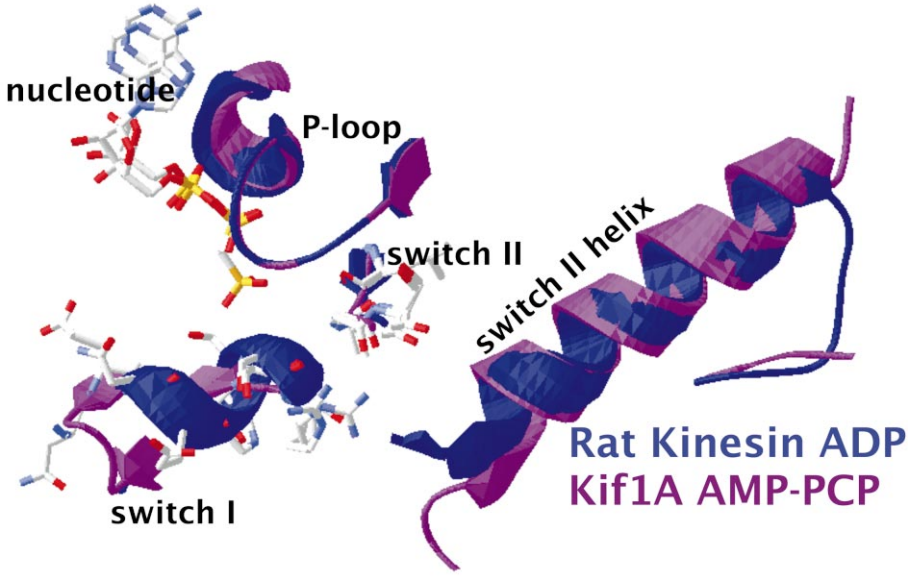

Fig. 3. Nucleotide binding pockets and switch II helices in Kif1A and rat conventional kinesin. X-ray models of the ADP-bound rat conventional kinesin (blue) and the AMP-PCP-bound Kif1A (pink) are superimposed on the basis of their P-loops. Although both structures presumably represent different nucleotide states, the switch II helices are located remarkably close. In contrast, in ADP-bound Kif1A, this helix displays a significant angular shift and resembles the human kinesin ADP conformation (not shown). Apparently, the switch II helix position is variable and may be coupled only loosely to the nucleotide state.

Table 2
Kar3p point mutations

Mutant	Location of mutation	Phenotype	Structural effect
Kar3p wild-type	–	–	Similar to previous KAR3 structure
Kar3p R598A	switch I; SSRSH→SSASH	weak microtubule binding	Initial part of switch I disordered; loop adjacent to switch II ordered
Kar3p E631A	switch II; LAGSE→LAGSA	strong microtubule binding	Switch I and II disordered
Kar3p N650K	switch II helix; INKS→IKKS	strong microtubule binding but no ADP release	Disruption of a salt bridge between switch II and switch II helix

microtubule binding behavior. For the E→A mutation in human kinesin, it is known that it leads to an inactivation of ATP hydrolysis and tight microtubule binding [22]. The mutant head, however, is still able to signal ATP binding to the second head of the kinesin dimer. Hence, on the one hand formation of the R–E salt bridge and the ordering of the switch regions may be required for hydrolysis and the release of kinesin from microtubules. On the other hand, there may be additional elements surrounding the ATP pocket that communicate the nucleotide state to the partner head.

The third Kar3p mutant (N→A in the switch II helix) disrupts a different intermediate state. Functionally, hydrolysis still works but the ADP-Kar3p does not release from the microtubule. Structurally, this mutant differs from wild-type in that a salt bridge between switch II and the switch II helix cannot be formed. Apparently, this salt bridge is required for the transition to the weak microtubule binding state, again arguing for the importance of the switch II helix in modulating microtubule affinity.

3. Conclusion

The crystal structures of several KRPs together with mutagenesis studies provide evidence for a conserved catalytic mechanism among all kinesins. The relation between the nucleotide state and microtubule affinity so far remains speculative as no filament-bound high-resolution structure has been obtained yet. The available data, however, hint at a pathway that involves a system of hydrogen bonds between the switch regions that can be rearranged during catalysis. In addition, the transmission of this rearrangement to the microtubule binding site via the switch II helix can be inferred. In addition, in conventional kinesins, the interdependence of nucleotide binding and microtubule affinity may be accompanied by a pathway that assures communication between the two heads during processive movement [22].

One of the remaining big questions is how the primary events in the motor core are converted into motility, force production, or microtubule depolymerization, respectively. Domains and motifs outside the motor domain may play a crucial role. In conventional kinesin, an organelle motor, the so-called neck-linker, a region that immediately follows the C-terminal end of the motor core, is known to be essential for motility [23]. In contrast, the crystal structure of the KRP Eg5, a mitotic kinesin, shows a completely different arrangement of the neck-linker. These different arrangements probably reflect different functions. Whereas conventional kinesins are designed to move processively, Eg5 may be used to hold force. Future studies are likely to reveal further elements of nature's motors toolbox.

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