Review

The role of microtubules in processive kinesin movement

Masahide Kikkawa

Graduate School of Science, Kyoto University, Oiwake, Kita-shirakawa, Sakyo-ku, Kyoto, 606-8502, Japan

Kinesins are microtubule-based motors that are important for various intracellular transport processes. To understand the mechanism of kinesin movement, X-ray crystallography has been used to study the atomic structures of kinesin. However, as crystal structures of kinesin alone accumulate, it is becoming clear that kinesin structures should also be investigated with the microtubule to understand the contribution of the microtubule track to the nucleotide-induced conformational changes of kinesin. Recently, several high-resolution structures of kinesin with microtubules were obtained using cryo-electron microscopy. Comparison with X-ray crystallographic structures revealed the importance of the microtubule in determining the conformation of kinesin. Together with recent biophysical data, we describe different structural models of processive kinesin movement and provide a framework for future experiments.

Introduction

Kinesin is a microtubule-based motor that uses ATP as the energy source. Kinesins are involved in various intracellular trafficking events such as axonal transport and chromosome segregation [1,2]. Several models have been proposed to explain the movement of conventional kinesin, such as symmetric hand-over-hand, asymmetric hand-over-hand and inchworm. Recent experiments have focused on distinguishing these models, and recent biophysical experiments favor the asymmetric hand-over hand model [3,4]. However, the track on which kinesin moves, the microtubule, is often considered as a passive track in these models. However, recent structural data derived from cryo-electron microscopy (cryo-EM) indicate that microtubules have a more active role. Here, we describe recent structural data and the current problems surrounding understanding the mechanism of kinesin movement, and discuss the role of kinesin–microtubule interactions.

The kinesin motor domain is similar to switching G proteins

The motor domain of kinesin is of great interest because it can efficiently convert chemical energy from ATP hydrolysis to mechanical movement (for the overall architecture of kinesins, see Box 1). Kinesin can generate 8-nm steps against a load of 7 pN using ~100 pN nm of chemical energy derived from hydrolysis of one ATP molecule (Here, we use pico-Newton nanometer, pN nm, per molecule as units of free energy according to Howard [5].) Therefore, ~50 pN nm of mechanical energy is generated and the energy conversion efficiency is close to 50%. To understand the highly efficient conversion mechanism, the structure and functions of the kinesin motor domains have been extensively studied. More than a decade ago, X-ray crystallography revealed that the structure of the kinesin motor domain is similar to that of myosins and GTP-binding proteins (G proteins) [6,7]. In G proteins, the 'switch region' can detect the presence (GTP) or absence (GDP) of γ-phosphate in the nucleotide and respond by undergoing conformational changes.

Is nucleotide state enough to 'switch' kinesin conformation?

As expected from the similarity between kinesins and G proteins, marked conformational changes have been observed within the 'switch regions' of kinesin motors using X-ray crystallography [8,9]. The first switch region consists of a single loop, L9, called the switch I loop [Figure 1a(ii)]. Like G proteins, this loop is close to the phosphate of the bound nucleotide and presumed to sense the presence (ATP) or absence (ADP) of a γ-phosphate. A second conformational change is observed around the switch II cluster that surrounds helix α4 [Figure 1a(ii)]. In plus-end directed motors, the movement of the kinesin switch II cluster is always correlated with the conformation of the third region, the neck linker [Figure 1a(ii)]. The neck linker is docked onto the kinesin catalytic core when the switch II helix is closer to the nucleotide [Figure 1a–c(ii)], whereas it is undocked or invisible when the switch II helix is closer to the β-sheet and in the same plane as the α6 helix [Figure 1a,b(i)]. The docking of the neck linker on the kinesin catalytic core presumably delivers a plus-end-directed pull on its cargo [8,10,11] (Figure 2). This model is called 'the neck-linker-docking model'.

A straightforward interpretation of the X-ray crystallographic structures of kinesin is that the energy-transmitting pathway starts from the γ-phosphate of the nucleotide, goes through the switch I loop and switch II helix, and reaches the neck linker. However, accumulating crystallographic data indicate that the nucleotide alone might not be enough to induce conformational switching. First, the conformations of switch I do not depend on nucleotide state. Second, a so-called 'ATP-like' structure has been observed in the presence of ADP [Figure 1c(ii)]. Similarly, an 'ADP-like' structure has also been observed in the presence of an ATP analog [12] [Figure 1c(i)]. Furthermore,

Corresponding author: Kikkawa, M. (mkikkawa@em.biophys.kyoto-u.ac.jp).

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Box 1. Architecture of kinesins

Conventional kinesin consists of two heavy chains and two light chains that form a dumbbell-shaped structure (Figure Ia). One kinesin molecule has two heads, a long coiled-coil stalk and a cargo-binding tail with light chains [53,54] (Figure Ib). The head domain, known as the 'motor' domain, has both ATPase- and microtubule-binding activities. The two heads are connected to the α-helical coiled-coil that forms the stalk domain (Figure Ic). The beginning of the stalk is called the 'neck' and a short (13 amino acid) polypeptide segment, the 'neck linker', connects the head and the neck (Figure Id).

According to the standardized nomenclature, kinesins are classified into 14 families [55] (Figure Ie), and domain organizations have been deduced using the SMART server [56]. Plus-end-directed kinesins have an N-terminal motor domain(s) and include a dimeric kinesin heavy chain (KHC; standardized name, Kinesin-1), monomeric KIF1A (Kinesin-3), Eg5 (Kinesin-5) and CENP-E (Kinesin-7). KIF2C (Kinesin-13) is a microtubule-depolymerizing kinesin, which has a centrally located kinesin motor domain. Minus-end-directed kinesins belong to the Kinesin-14 family, and include Ncd, Kar3 and kinesin-like calmodulin-binding protein (KCBP), and their motor domains are located at the C-terminal region.

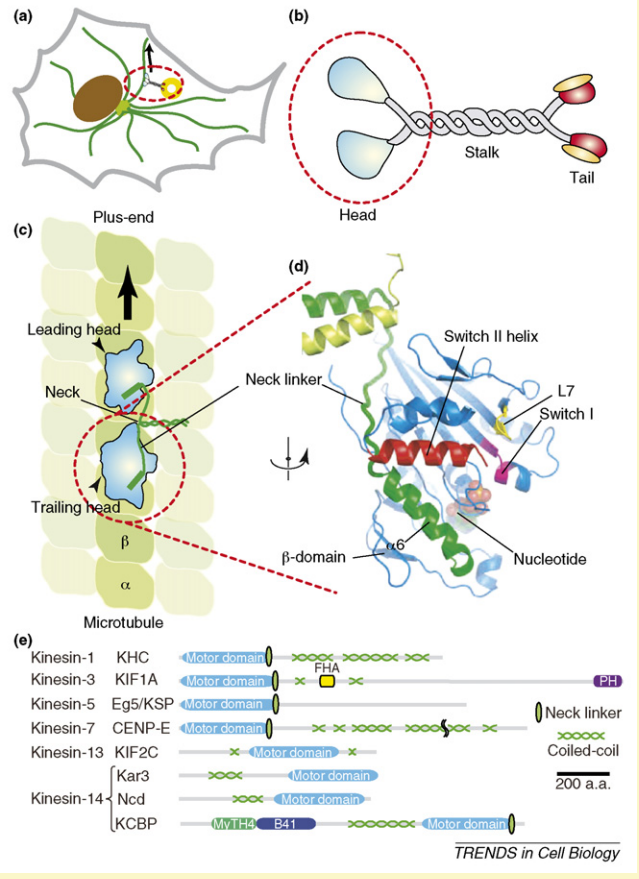

Figure I. Architecture of kinesins. **(a)** Kinesins are involved in various cargo transports in cells. **(b)** Schematics of the overall structure and **(c)** two-head-bound state of conventional kinesin are shown. **(d)** The atomic structure of kinesin head viewed from the microtubule binding surface. Domains involved in the conformational changes are labeled. **(e)** The primary domain organization of kinesins for which crystal structures are available.

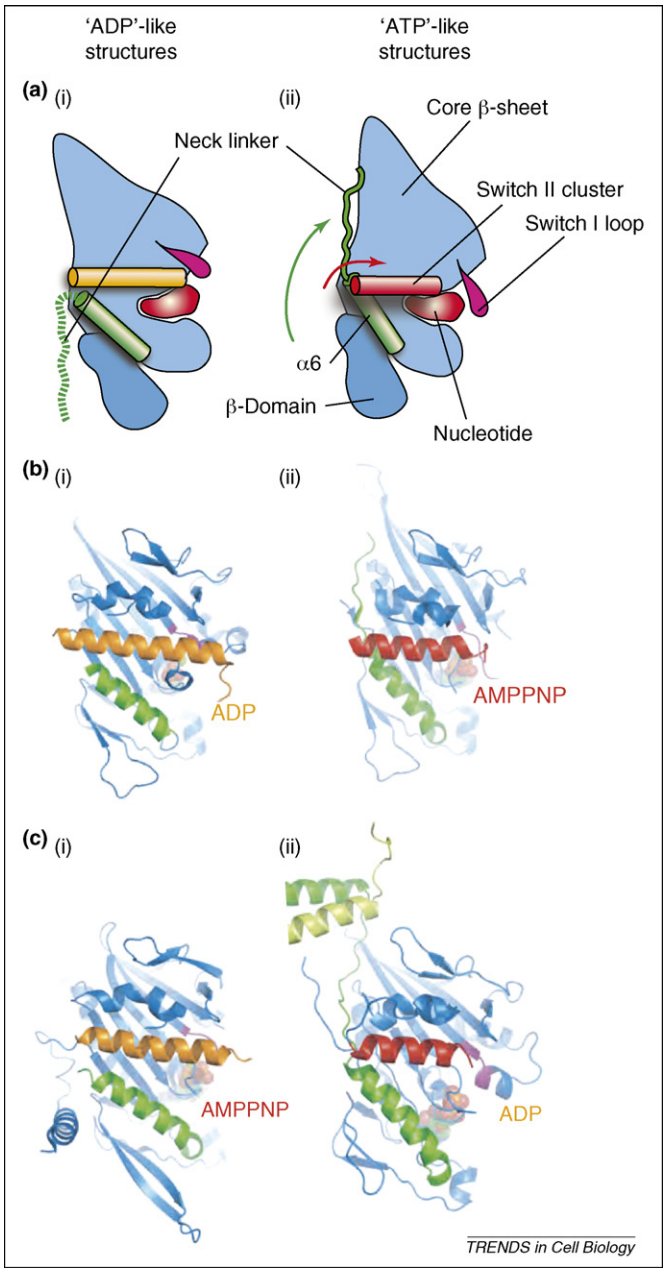

Figure 1. Is nucleotide state enough to switch the conformation of kinesin? Kinesin structures are approximately classified into two groups: so called 'ADP'-like and 'ATP'-like structures. Contrary to the names, these two structures are not always correlated with the bound nucleotide in the X-ray crystallographic structures. **(a)** Schematic structures of the kinesin motor domain. The ADP-like (i) and ATP-like (ii) structures are distinguished by the relative arrangement between the switch II cluster and the core β-sheet. In the ADP-like structure, the switch II cluster is closer to the β-sheet and in the same plane as the α6 helix. In the ATP-like structure, the helix moves towards the nucleotide like a 'piston' and the distance from the core β-sheet is relatively greater. In most 'ADP'-like crystal structures, the switch II helix is longer than that of the 'ATP'-like structures; however, this might not be true in the microtubule-bound state because a longer switch II helix has also been observed in the AMPPNP state [25]. **(b)** Examples of crystal structures of kinesin. (i) KIF1A (monomeric plus-end-directed kinesin) in the ADP state (PDB: 1I5S [8]). (ii) KIF1A in the AMPPNP state (PDB: 1VFV [16]). **(e)** Examples of kinesins, the structures of which do not match with the bound nucleotide: (i) KIF2C (PDB: 1V8K [12]) with bound AMPPNP, crystallized in the ADP-like form. (ii) Rat conventional kinesin with ADP (PDB: 2KIN [14]), crystallized in the ATP-like form.

recent extensive multivariate analyses of 37 available atomic structures from five different kinesin subfamilies identified the switch II cluster (α4–loop12–α5–loop13) as the first principle component of conformational differences, but failed to support tight coupling between nucleotide state and kinesin conformation [13]. In summary, there are two principle conformations of the switch II cluster in solution and crystals, but they are interchangeable in solution and independent of nucleotide state [9].

Examination of the available atomic structures of kinesins implies that the pathway that induces conformational