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Subunits interactions in kinesin motors

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Abstract

Kinesins form a large and diverse superfamily of proteins involved in numerous important cellular processes. The majority of them are molecular motors moving along microtubules. Conversion of chemical energy into mechanical work is accomplished in a sequence of events involving both biochemical and conformational alternation of the motor structure called the mechanochemical cycle. Different members of the kinesin superfamily can either perform their function in large groups or act as single molecules. Conventional kinesin, a member of the kinesin-1 subfamily, exemplifies the second type of motor which requires tight coordination of the mechanochemical cycle in two identical subunits to accomplish processive movement toward the microtubule plus end. Recent results strongly support an asymmetric hand-over-hand model of ''walking'' for this protein. Conformational strain between two subunits at the stage of the cycle where both heads are attached to the microtubule seems to be a major factor in intersubunit coordination, although molecular and kinetic details of this phenomenon are not yet deciphered. We discuss also current knowledge concerning intersubunit coordination in other kinesin subfamilies. Members of the kinesin-3 class use at least three different mechanisms of movement and can translocate in monomeric or dimeric forms. It is not known to what extent intersubunit coordination takes place in Ncd, a dimeric member of the kinesin-14 subfamily which, unlike conventional kinesin, exercises a power-stroke toward the microtubule minus end. Eg5, a member of the kinesin-5 subfamily is a homotetrameric protein with two kinesin-1-like dimeric halves controlled by their relative orientation on two microtubules. It seems that diversity of subunit organization, quaternary structures and cellular functions in the kinesin superfamily are reflected also by the divergent extent and mechanism of intersubunit coordination during kinesin movement along microtubules.

Keywords: Kinesin; Molecular motor; Microtubule; Processivity; Dimer; Strain

Introduction

Molecular motors are indispensable for numerous essential cellular processes such as organelle movement, axonal transport, spindle formation and maintenance, microtubule polymerization dynamics, etc. All parameters for a particular kind of transport (timing, kind of cargo, localization of cargo entry and leaving point, velocity, processivity) have to be precisely controlled since they are crucial for precision of the transport itself. One of the means of tuning up the transport machinery to a particular task is a great diversity of molecular motors; proteins specialized in converting chemical energy into mechanical work. Recent progress in the

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elucidation of the molecular mechanisms underlying motor protein function had an important impact on our understanding of intracellular transport at the cellular level. The three linear motors: myosin, kinesin and dynein are in most cases dimeric proteins that have two catalytic domains, named "heads" connected by a coiled-coil "stalk". Motors are different from regular enzymes since in motors the mechanical cycle is tightly coupled to the chemical reaction that takes place at the catalytic pocket of the protein. Each motor also undergoes a third cycle: binding and releasing of its track (microtubule or F-actin). Since molecular motors move in discrete steps, proteins that transport cargoes alone or in a small group (Fig. 1a) must coordinate their heads in such a way as to prevent dissociation of both domains from the track. Motors that work in large groups often participate in well-organized arrays. Fig. 1b–d shows several common situations in which multiple motors translocate particles (Fig. 1b), move a microtubule or actin filament (Fig. 1c) or organize a mitotic or meiotic spindle (Fig. 1d). Motors belonging to the first group, i.e. acting alone, are sometimes called "porters", the second "rowers" (Leibler and Huse, 1993). Clearly, it is important for a porter to control its heads during the entire mechanochemical cycle. Otherwise, the diffusion will carry the motor with its cargo away from the track. Thus, a porter motor will move "processively" along the track, i.e. will translocate without detachment for many cycles, hydrolyzing 1 molecule of ATP at each step. Logically, a porter kinesin should spend a considerable fraction of its cycle in the microtubule-bound state. This parameter is called the "duty ratio" (Howard, 1997). For the highly processive conventional kinesin, the duty ratio is $\geqslant 0.5$ (Howard, 1997). In contrast, a rower kinesin, such as minus end-directed Ncd, will execute its force-generating step quickly, and the rest of its cycle will be spend in the unbound form. The duty ratio for Ncd is about 0.08 (Pechatnikova and Taylor, 1999). Fig. 1b shows a large vesicle or organelle which is translocated by several motors. Although the motors may be non-processive, an ensemble of motors can transport a cargo in a processive fashion because there is a probability that at least one motor will keep the cargo from falling off the microtubule. A recent theoretical study indicates that the distance for which the cargo is moved without detaching from the tracks depends on the number of motors participating in the transport. Ten kinesin molecules can translocate cargoes in the centimeter range (Klumpp and Lipowsky, 2005). If the cargo is pulled against an external load this force will be shared between the motors providing a non-trivial motor–motor coupling and a nonlinear force–velocity relationship (Klumpp and Lipowsky, 2005).

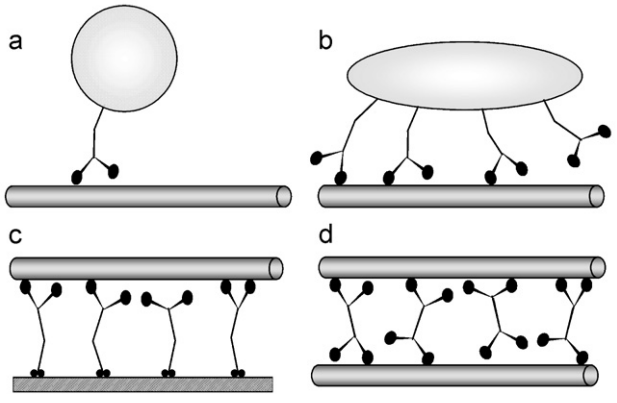

Fig. 1. Multiple modes of cellular transport and force generation used by molecular motors. (a) A single motor protein, for example kinesin-1, is carrying a cargo. To perform such function the motor must be processive. (b) A group of motors are transporting an organelle, for example a mitochondrion. In this case, individual motors may be non-processive but the particle is being moved processively. (c) Multiple motors, attached to a solid support slide a microtubule. Such situation exists during in vitro motility assays but also in the cell (e.g., Ncd). (d) Similar to (c) but with a bipolar kinesin, such as Eg5, that performs relative sliding of two microtubules.

Techniques

Progress of our understanding of kinesin coordination has strongly depended on the introduction of specialized techniques. One line of research involved careful kinetic studies of natural homodimeric kinesin constructs and interpretation of the results in light of different kinetic and biophysical models describing coordination of the chemical and mechanical events. It seems, however, that recent advances in this area resulted mainly from the use of a specialized design of the study object, quasi-heterodimeric constructs obtained with protein engineering techniques where the two subunits of kinesin differ by rationally designed single amino-acid substitutions (Kaseda et al., 2002, 2003; Skowronek and Kasprzak, 2002; Higuchi et al., 2004; Klumpp et al., 2004; Endres et al., 2006). Such constructs make possible to discriminate between the subunits and provide insight into influence of one subunit on the activity of the dimer. Another use of quasi-heterodimeric kinesins is specific fluorescent labeling of a single subunit (Tomishige et al., 2006; Yildiz et al., 2004b). Quasi-heterodimeric kinesins have been studied in many commonly used assays, such as biochemical ATPase assay, in vitro motility assay, as well as displacement and force measurements with the laser trap.

Research in the field has also substantially benefited from a development of physicochemical techniques designed to follow the elementary steps of the force generation process. In solution, the mechanochemical cycles of kinesin motors are not synchronized. Their