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Torque Generation of Kinesin Motors Is Governed by the Stability of the Neck Domain

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SUMMARY

In long-range transport of cargo, prototypical kinesin-1 steps along a single protofilament on the microtubule, an astonishing behavior given the number of theoretically available binding sites on adjacent protofilaments. Using a laser trap assay, we analyzed the trajectories of several representatives from the kinesin-2 class on freely suspended microtubules. In stark contrast to kinesin-1, these motors display a wide range of left-handed spiraling around microtubules and thus generate torque during cargo transport. We provide direct evidence that kinesin's neck region determines the torque-generating properties. A model system based on kinesin-1 corroborates this result: disrupting the stability of the neck by inserting flexible peptide stretches resulted in pronounced left-handed spiraling. Mimicking neck stability by crosslinking significantly reduced the spiraling of the motor up to the point of protofilament tracking. Finally, we present a model that explains the physical basis of kinesin's spiraling around the microtubule.

INTRODUCTION

To ensure an efficient intracellular transport of cargo, eukaryotic cells developed mechanisms of directed transport. One of these mechanisms involves processive molecular motors, which move along the cytoskeletal filaments over long distances by coupling the ATP hydrolysis to mechanical work. Once attached, processive motors can thus take multiple "steps" on their respective filaments without detaching; conversely, unprocessive motors detach from their filaments after one "step." Directed transport by molecular motors plays essential roles in diverse cellular processes, e.g., ciliary transport, transport of membrane-bound vesicles in the cytoplasm, or chromosome segregation during mitosis.

Numerous members of the myosin, kinesin, and dynein families unidirectionally translocate along their filamentous tracks actin and microtubules. Based on this ability, they are termed linear motors. However, these molecules do not always follow a strict linear path along their track; they are capable of producing force perpendicular to their direction of motion as well. The first molecular motor discovered to generate torque was the single-headed dynein purified from Tetrahymena cilia. In in vitro motility assays, surface-attached dynein motors rotated the microtubules around their axis while translocating them in a linear fashion (Vale and Toyoshima, 1988). Such torsional force in addition to axial force generation was subsequently displayed by representatives from all three superfamilies of molecular motors (Nishizaka et al., 1993; Walker et al., 1990; Yajima and Cross, 2005; Yajima et al., 2008).

Within the kinesin superfamily, different behaviors are observed with respect to torque generating properties. Processive kinesin-1, for example, tracks precisely a single protofilament while stepping along a microtubule (Ray et al., 1993). This means that on a microtubule composed of 13 protofilaments where the protofilament axis is aligned with the microtubule axis, tracking results in a perfectly straight motion. In contrast, the weakly processive mitotic kinesin Eg5 (Valentine et al., 2006) follows a left-handed helical path with a pitch of ~2 μm (i.e., it rotates counter-clockwise, as observed with respect to its walking direction), which coincides neither with a possible supertwist of protofilaments nor with any of the helices of the tubulin lattice (Yajima et al., 2008). Lastly, unprocessive kinesins such as dimeric Ncd or artificially single-headed kinesin-1 or kinesin-5 constructs produce a pronounced left-handed microtubule rotation with a pitch of 0.3 μm (Walker et al., 1990; Yajima and Cross, 2005; Yajima et al., 2008). These findings have led to the proposal that the degree of torque generation might serve as a measure for processivity or lack thereof (Yajima et al., 2008).

The latest addition to the list of torque-generating kinesins is the heteromeric kinesin (kinesin-2 family) of *C. elegans* (Pan et al., 2010). Heteromeric kinesins are unique among double-headed motors in that they combine two distinct catalytic subunits to generate a functional motor. This heterodimeric motor associates C-terminally with the nonmotor subunit KAP (Kinesin Associated Protein) to form a heterotrimer (Vukajlovic et al., 2011; Wedaman et al., 1996; Yamazaki et al., 1996). Interestingly, the kinesin-2 from *C. elegans* pairs an unprocessive subunit, KLP11, with a processive one, KLP20, to constitute a processive motor (Brunnbauer et al., 2010). In apparent agreement with the aforementioned hypothesis (Yajima et al., 2008), the unprocessive subunit KLP11, but not the processive KLP20 subunit, produced torque (Pan et al., 2010). Currently, no molecular mechanism exists to explain how kinesins generate torsional in addition to axial force. Is the propensity to generate

torsional force indeed an indicator of processivity, and is therefore torque generation indeed only observed with unprocessive or weakly processive kinesins? And above all, how is torque produced mechanistically?

So far, all results obtained on kinesin's torque-generating properties are inferred from observations of sliding microtubules on surface-attached motors (Nitzsche et al., 2008; Pan et al., 2010; Ray et al., 1993; Walker et al., 1990; Yajima and Cross, 2005; Yajima et al., 2008). Here, we have employed a laser trap assay (Ali et al., 2002, 2004) that allows the tracking of kinesin motion on suspended microtubules between two trapped beads in solution. This experimental geometry is not only a closer mimic of cargo transport but also provides a direct read-out on the motor's torsional pitch. We applied this assay to a range of heterodimeric kinesin-2 motors involved in distinct transport processes in the cytoplasm as well as in cilia. Unexpectedly, we found that heterodimeric kinesin-2 of diverse organisms display an astonishingly broad range of pitches along their paths on microtubules. Thus, torque generation is not confined to mostly artificial, unprocessive, or weakly processive members of the kinesin family but seems to be a prevalent feature for natural kinesin motors involved in diverse transport processes. To identify the domain(s) that determine such behavior, we generated a series of chimeric constructs using the processive kinesin-2 motors from *C. elegans* and mouse (Brunnbauer et al., 2010; Muthukrishnan et al., 2009). Our dissection reveals that the neck, but not the neck linker or the head domains, dictates the spiraling behavior of a motor. Unequivocal support for this finding comes from experiments where a crosslink between the neck linker and the neck of processive kinesin-1 acts as a molecular switch: tampering with the neck stability by introducing flexible residues leads to strong torque generation; mimicking neck stability by crosslinking constrains the motor's path up to the point of single protofilament tracking. In an equivalent approach, we introduced reactive cysteines into the neck region of the kinesin-2 motor from sea urchin that displays the strongest spiraling around the microtubule. The subsequent crosslinking, which again mimics a stable neck, significantly reduced the motor's propensity to generate torque. Based on the structure of the kinesin-1 motor available at atomic resolution (Kozielski et al., 1997; Sindelar et al., 2002; Sindelar and Downing, 2007), we provide a simple mechanistic model that accommodates the correlation between the structure of the neck and the propensity to generate torque in kinesin-1.

RESULTS

Probing the Three-Dimensional Trajectories of Kinesin Motors along Suspended Microtubules

We used a multiple-beam optical tweezers setup to study the path of kinesin motors in an unconstrained geometry that allows the motors to access the entire microtubule surface (Ali et al., 2002, 2004). Individual biotinylated and fluorescently labeled microtubules were suspended between two streptavidin-coated "pillar" beads with 3 μm in diameter, which were trapped approximately 20 μm apart. A third trapping potential was used to capture a smaller "cargo" bead with 1 μm in diameter that was coated with motor molecules. The third trap was used to steer the cargo bead and establish contact with the suspended microtubule. As soon as the motors started walking along the microtubule, the cargo bead was released, and its motion along the microtubule was monitored via bright-field microscopy (Figure 1A). Specifics of the experimental geometry and the data analysis are discussed in the Supplemental Information (Figure S1).

In the first set of experiments, we established the robustness of our experimental geometry using kinesin-1 from *D. melanogaster* (*Dm*KHC) (Figure 1B), which is known to track a single protofilament on the microtubule surface (Nitzsche et al., 2008). When we tested the movement of *Dm*KHC-coated beads on different suspended microtubules, we mainly observed a left-handed spiraling motion of the beads around the microtubule with a mean pitch of 5.7 ± 1.1 μm (mean ± SD, 17 beads tested on 7 different microtubules) (Figures 1C and 1D and Movie S1). This value matches closely the predicted protofilament supertwist of a microtubule composed of 14 protofilaments according to the lattice rotation model (Chrétien and Wade, 1991; Ray et al., 1993). Furthermore, we occasionally detected straight movement or a right-handed spiraling (pitch ~4 μm), which correspond to microtubules built from 13 or 12 protofilaments, respectively (Figure 1D). Taken together, our results are consistent with *Dm*KHC tracking individual protofilaments as demonstrated previously and show that, as expected for the polymerization conditions used here, 14-protofilament microtubules constitute the majority (Ray et al., 1993).

Heterodimeric Kinesin-2 of Diverse Organisms Display an Astonishing Variability in Their Trajectories on Microtubules

Heteromeric kinesins coevolved with the cilia to work on microtubule doublets and were later adapted for cytoplasmic transport on singlet microtubules (Mitchell, 2007; Scholey, 2003). The neuronal transporter *Mm*KIF3a/3b (Yamazaki et al., 1995) and the melanosome transporter *Xl*KLP3a/3b (Tuma et al., 1998) are the prominent representatives of kinesin-2 motors involved in cytoplasmic transport. Processive movement along microtubules was demonstrated with *Mm*KIF3a/3b, *Xl*KLP3a/3b, and *Ce*KLP11/20 (Brunnbauer et al., 2010; Kural et al., 2007; Muthukrishnan et al., 2009), whereas *Sp*KRP85/95 was reported to be a nonprocessive motor (Pierce et al., 1999).

We probed the transport paths of the aforementioned full-length heterodimeric kinesin-2 motors in our setup. Because the wild-type *Ce*KLP11/20 is an autoinhibited motor, we introduced the corresponding deinhibiting mutations in the conserved region within the stalk (see Supplemental Information and Figure S2A) of all the kinesin-2 motors to circumvent potential experimental problems caused by inhibited motors (Brunnbauer et al., 2010; Imanishi et al., 2006). The trajectories of these different kinesin-2 constructs along suspended microtubules revealed a tremendous variability in their spiraling behavior. Except for *Mm*KIF3a/3b, all heterodimers exhibited a characteristic left-handed spiraling (Figure 2 and Movie S2). Equivalent results were obtained with all constructs containing the wild-type stalk, demonstrating that the deinhibiting mutations do not interfere with the propensity of the motors to generate torque (Figures S2B and S2C). *Sp*KRP85/95 displayed the tightest pitch