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ATPase Cycle of the Nonmotile Kinesin NOD Allows Microtubule End Tracking and Drives Chromosome Movement

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SUMMARY

Segregation of nonexchange chromosomes during *Drosophila melanogaster* meiosis requires the proper function of NOD, a nonmotile kinesin-10. We have determined the X-ray crystal structure of the NOD catalytic domain in the ADP- and AMPPNP-bound states. These structures reveal an alternate conformation of the microtubule binding region as well as a nucleotide-sensitive relay of hydrogen bonds at the active site. Additionally, a cryo-electron microscopy reconstruction of the nucleotide-free microtubule-NOD complex shows an atypical binding orientation. Thermodynamic studies show that NOD binds tightly to microtubules in the nucleotide-free state, yet other nucleotide states, including AMPPNP, are weakened. Our pre-steady-state kinetic analysis demonstrates that NOD interaction with microtubules occurs slowly with weak activation of ADP product release. Upon rapid substrate binding, NOD detaches from the microtubule prior to the rate-limiting step of ATP hydrolysis, which is also atypical for a kinesin. We propose a model for NOD's microtubule plus-end tracking that drives chromosome movement.

INTRODUCTION

Proper and faithful segregation of chromosomes during cell division is an essential biological process for normal eukaryotic life. During meiosis, the segregation of those chromosomes that fail to undergo meiotic exchange (also known as achiasmate or nonexchange chromosomes) is accomplished by the so-called "distributive system" of meiotic segregation. Distributive segregation systems have been well characterized in numerous organisms, including budding yeast, *C. elegans*, and *D. melanogaster* (Cheslock et al., 2005; Dernburg et al., 1996; Meneely et al., 2002). The *nod* gene was first identified on the basis of a mutant that strongly affected the segregation of homologous chromosomes at the first meiotic division in *Drosophila* oocytes (Carpenter, 1973). Specifically, the protein product of the nod^{+} gene is required for the proper segregation of nonexchange chromosomes without impairing the segregation of chromosomes that do undergo meiotic exchange via crossing over.

NOD is a chromokinesin-like protein from the kinesin-10 family that is localized along the arms of meiotic chromosomes (Afshar et al., 1995; Zhang et al., 1990). NOD consists of an N-terminal kinesin-like catalytic domain and a C terminus that contains two types of DNA binding motifs (Cui and Hawley, 2005). Although NOD lacks the capacity for movement along microtubules (MTs) (Matthies et al., 2001), it binds preferentially to MT plus ends both in vivo and in vitro and stimulates MT polymerization (Cui et al., 2005). These results suggest that NOD functions by tethering the chromosome arms to polymerizing MT plus ends, thus "pushing" the chromosome arms away from the poles and toward the metaphase plate (Matthies et al., 1999). This model was independently supported in mitotic cells through RNAi-based elimination of NOD function (Goshima and Vale, 2003).

The finding that ATP hydrolysis was not required for NOD to stimulate MT polymerization raised concerns about the role of the catalytic domain in mediating NOD function (Cui et al., 2005). Genetic studies have identified a dominant cold-sensitive allele of *nod,* known as nod^{DTW}, which is associated with a substitution near the active site (S94N). As a heterozygote, the nod^{DTW} mutant exhibits severe defects in chromosome segregation in female meiosis, which fully mimic those observed for loss-of-function alleles. This demonstrates that the NOD^{DTW} mutant protein not only fails to function itself, but can also antagonize the function of wild-type NOD. Although loss-of-function alleles of *nod* have no detectable effects on mitosis, the nod^{DTW} mutation induces a temperature-sensitive defect in mitosis that leads to cold-sensitive lethality. The dominant negative effects of the nod^{DTW} mutation can be ablated by second-site amino acid substitutions within the *nod* gene (Rasooly et al., 1994). Although these second-site intragenic mutations are not true second-site revertants and do not restore wild-type function, they do abolish the deleterious function of the NOD^{DTW} mutant. One such mutation (D151N) is adjacent to the MT binding region,

while another (R194H) is involved in the communication between the active site and the MT binding region. These data suggest that MT binding most likely can modulate NOD's ATPase cycle.

Cytoskeletal motor proteins from the kinesin superfamily are enzymes that utilize ATP hydrolysis to perform various functions in eukaryotic cells. Their catalytic domain coordinates movements of conserved structural elements located at the active site (phosphate binding loop [P loop consensus: GQTxxGKT/S], Switch 1 [Sw1: NxxSSR], Switch 2 [Sw2: DxxGxE]) with the MT-binding interface (Kull and Endow, 2002; Vale, 2003). The structural differences among kinesins result in variations of the rate and equilibrium constants that govern their ATPase cycles. Therefore, each motor elicits a different work output that is utilized to perform different tasks inside cells.

Although the detailed structural mechanism of kinesin activity remains unknown, the nucleotide state at the active site is thought to trigger a switch in conformation that is then transmitted to the adjacent regions of the core that interact with the MT. This results in changes in MT binding affinity and stabilization of the neck linker in alternate conformations (Kikkawa et al., 2001; Kull and Endow, 2002). The structural elements of kinesins that interact with the MT include the β5-L8 lobe consisting of two antiparallel β strands in L8 (β5a and β5b), L11, and the "Sw2 cluster" that comprises the relay helix α4, L12, and α5 (Sosa et al., 1997; Woehlke et al., 1997). Communication from the active site to the MT binding region is likely accomplished through two pathways: (1) Sw1 (L9) to α3 to β5-L8 lobe (Ogawa et al., 2004), and (2) Sw2 (L11) to the relay helix α4 to the remainder of the "Sw2 cluster," which dictates the orientation of the neck linker for directed force production (Vale and Milligan, 2000).

Here, we present a minimal ATPase mechanism of the NOD catalytic domain. We have determined the crystal structures of NOD bound to adenosine diphosphate (ADP) and adenosine 5′-(β,γ-imido)triphosphate (AMPPNP, a nonhydrolyzable ATP analog) as well as generated a cryo-electron microscopy (cryo-EM) reconstruction of the MT•NOD complex in the nucleotide-free state. We have used kinetic and thermodynamic methodologies to characterize the key steps in the NOD ATPase cycle. Our mechanistic analysis reveals that NOD binds tightly to MTs in the nucleotide-free state, yet other nucleotide states including AMPPNP are significantly weakened. Rapid substrate binding leads to NOD detachment from the MT prior to ATP hydrolysis. In the absence and presence of MTs, ATP hydrolysis is the rate-limiting step, which is different from other kinesins. Taken together, these studies suggest a model for how a nonmotile kinesin tracks MT plus ends and harnesses the force of MT polymerization to drive the movement of chromosome arms.

RESULTS

Structural Comparison of NOD with Other Kinesins

We solved the X-ray crystal structure of the NOD catalytic domain in the ADP and AMPPNP states (Figures 1A and 1B, and Figure S1 available online). The crystallographic model for NOD•ADP was refined to 1.9 Å and NOD•AMPPNP to 2.5 Å (Table S1). When the two NOD structures were superposed using the P loop, the root mean square deviation (RMSD) between 287 alpha carbons was 1.62 Å (Figures 1C and 1D). The most dramatic differences between the ADP and AMPPNP states were observed in the loops of the MT binding region (L11 and L8), helix α3, and the loops containing Sw1 (L9) and Sw2 (L11). Both NOD structures were systematically compared to the 49 kinesins in the Protein Data Bank (PDB, May 2008), with the P loop used to superpose the structures. This superposition showed that the core β sheet was similar, with the exception of the length of β6 and β7 being 1–4 amino acids shorter in NOD. Large structural differences were also observed among the loops and helices that surround the core: L5, L8, L9-α3, L11-α4-L12-α5, and α6.

Nucleotide-Sensitive Relay between Sw1, Sw2, P Loop, and Nucleotide

A remarkable transition occurs in the hydrogen bonding pattern of well-conserved residues at the nucleotide binding site when NOD•ADP is compared with NOD•AMPPNP (Figures 1E and 1F). For NOD•ADP (Figure 1E), the absence of the γ-phosphate allows T89 in the P loop to form a hydrogen bond with E231 from Sw2. The positioning of the R204 from Sw1 allows for two hydrogen bonds with the backbone carboxyl groups of the conserved G229 and E231 residues in Sw2. As Sw1 S203 is 14.9 Å from Mg^{2+} and the amide group of Sw2 G229 is ~4.2 Å from where the location of the γ-phosphate would be in the ATP bound state, both Sw1 and Sw2 are in the open conformation.

For NOD•AMPPNP (Figure 1F), we observed the first fully closed conformation of Sw2 in any kinesin as defined by a direct hydrogen bond between the amide group of G229 and the γ-phosphate. Although L11 was mostly disordered in both nucleotide states, the visible portions at the base of the loop showed a distinct retraction away from the MT interface in NOD•AMPPNP compared to NOD•ADP (Figure 1D). This movement is likely the result of the fully closed conformation of Sw2 and appears to be stabilized by a salt bridge between R234 on L11 and E306 on α6 (Figure S2). In addition to the closure of Sw2, the presence of the γ-phosphate results in a conformational change in P loop T89 such that it forms a direct hydrogen bond to the γ-phosphate. E231 from Sw2 swings away from T89 and forms a hydrogen bond with S203 from Sw1, and R204 is reoriented toward the nucleotide pocket, where it interacts with one of the Mg-coordinated water molecules.

Since the amino acids involved in this hydrogen bond relay are well conserved in kinesins, myosins, and G proteins, we propose that the configuration of hydrogen bonding in each NOD structure represents two different intermediate states in the ATPase cycle. NOD•AMPPNP represents a structural intermediate that occurs after tight substrate binding, yet this state does not represent the "hydrolysis-competent" state given the open Sw1 (S203 = 12.3 Å from Mg^{2+}). Once Sw1 reaches the closed conformation, NOD can proceed along the pathway toward ATP hydrolysis. After P_i product is released, Sw1 and Sw2 can assume the configuration observed in the NOD•ADP structure.

NOD's L5 Directly Interacts with α3

Loop L5 appears to undergo conformational changes during the kinesin-5 ATPase cycle and interacts with monastrol-like