

The Role of ATP Hydrolysis at Distinct ATP Binding Sites in Cytoplasmic Dynein

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Dynein is a member of the AAA+ superfamily of ATPases with a characteristic ring structure composed of four functional ATP binding modules. Dynein differs from the other cytoskeletal motor proteins, kinesin and myosin, which both contain a single ATP binding site per motor domain. To elucidate the role of dynein's multiple nucleotide binding sites, we expressed a panel of recombinant dynein dimers in *S. cerevisiae* predicted to block ATP hydrolysis in each of dynein's 4 conserved AAA+ domains. Minimal functional dimers were created by fusing the motor domain to glutathione-S-transferase (GST), which forms a stable homodimer. For each mutant, we assayed the motor function by ATPase activity, speed in a multiple motor microtubule gliding assay, and processivity and speed of single dynein dimers in a total internal reflection- based single molecule motility assay. As expected, mutation of AAA1 blocks dynein motor activity. Surprisingly, we find that mutation of AAA4 dramatically enhances dynein processivity, but has no effect on the motor's single molecule velocity. Our results suggest that the AAA4 mutant has a higher affinity for microtubules, indicating that nucleotide hydrolysis at this site functions to coordinate dynein's microtubule association with its mechanochemical cycle.