THE EFFECTS OF REMOVAL OF C-TERMINI OF TUBULIN ON THE MITOTIC KINESIN CENP-E BINDING AND MICROTUBULE GLIDING

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ABSTRACT

Both α- and β-tubulin contain helices H11 and H12 located on the outer surface of the microtubule (MT). H12 extends into a disordered region that is characterized by a highly negatively charged motif of approximately 10-18 amino acid residues called the C-terminal tail (CTT). These MT CTTs have been shown to modulate the MT interactions of kinesin molecular motors for function.

The research presented here tests the hypothesis that the MT CTTs modulate the behavior of the kinesin motor CENP-E for its functions during chromosome congression, including ATP-promoted motility. The protease, subtilisin was used to remove the CTTs of α- and β-tubulin, and the impact of the loss of the CTTs was evaluated by MT•CENP-E co-sedimentation, CENP-E promoted MT gliding, and steady-state ATPase assays.

The results show that when 1 mM MgAMPPNP was used to mimic the CENP-E•ATP state, there was little difference in the binding affinity of the native MTs (MT_N) or subtilisin-treated MTs (MT_S). In contrast, when 1 mM MgADP was used to generate the CENP-E•ADP state, there was a significant decrease in fractional binding for both the MT_N and MT_S. The *in vitro* motility assays revealed an increase in the rate of CENP-E promoted MT gliding on subtilisin MTs, but a decrease in the persistence of CENP-E promoted MT gliding, resulting in almost complete detachment of subtilisin MTs from microscope coverslip. Furthermore, steady-state ATPase kinetics revealed a higher rate for ATP turnover for MT_S, consistent with the motility results. Together the results indicate that the normal MT•CENP-E force generation is mediated in part through electrostatic interactions between the CENP-E motor domain and the MT CTTs.