The C-terminus of kinesin-14 Ncd is a crucial component of the force generating mechanism

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Ncd is a homodimeric microtubule (MT) motor of identical subunits each comprising 700 amino acids. It is thought that members of kinesin-14 family use the power stroke, a lever-like pivoting action of a long and stiff element to exert force and movement. It has been suggested that the Ncd C-terminus (the last ~30 aa) is in some way involved in this process. To better understand the role of these amino acids we isolated 3 Ncd mutants: Ncd670 (aa 250-670), Ncd679 (250-679) and Ncd700SG(250-700, with the segment 671-679 replaced by a flexible linker consisting of -Ser-Gly- sequences). For these proteins we measured the affinity to MT, steady-state MT-activated ATPase and the gliding velocity in multiple motor assays. The mutations had a dramatic effect on all three parameters measured. The gliding speed of Ncd679 was ~25% of the WT, for the remaining two mutants it was <1% of the WT. While changes in the ATPase were slightly less pronounced (decrease to 25% of WT), the most surprising was a remarkably low, 10-50 times, affinity of the mutants to MT in the absence of nucleotides, suggesting that the C-terminal residues of Ncd play a role in modulating the interaction of the motor with MT.