Title:	Characterizing the Role of Hook2 as a Dynein Adaptor
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Abstract:	Spatio-temporal distribution of cellular organelles is mediated by motor proteins (dynein, kinesin and myosin) that drive a variety of intricate and important movements inside the cell. Molecular motors are linked to different cargo through adaptor proteins that not only aid in cargo selection but also enhance the processivity of motor. Cytoplasmic dynein regulates retrograde transport by associating with its activator dynactin. Previous studies in lower eukaryotes have implicated the role of Hook protein as dynein adaptors. Mammalian Hook proteins (Hook1 and Hook3) have been reported to mediate minus-end directed motility of early endosomes and organization of golgi complex respectively. However, the role of another Hook paralog, Hook2 that localizes to centrosomes, as a dynein adaptor is not yet known. Here we demonstrate that Hook2 directly binds to dynein light intermediate chain (LIC1) through its N-terminal Hook Superfamily Domain (HSD) while the first coiled coil (CC1) further strengthens this association. Further we show that similar to other adaptor proteins and hook paralogs, Hook2 also associates with different subunits of dynein (DIC) and dynactin (p150glued) complex. Point mutations in conserved residues of HSD abrogate these interactions. Interestingly, we find that the HSD and CC1 domain deletion mutant of Hook2 fails to associate with DIC but continues to bind to p150glued, suggesting that the association of Hook2 with dynactin could be independent of its association with dynein. Notably, we also found that amongst the three mammalian Hook proteins, Hook2 has the strongest affinity for endogenous DIC and p150. While characterizing the role of Hook2 in cell cycle, we have found putative Polo-like Kinase (PLK1) phosphorylation site in the CC1 domain of Hook2.
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