Title
Regulation of motor proteins by tubulin carboxy-terminal tails.

Authors
Sirajuddin, M
Rice, LM
Vale, RD

Publication Date
2013

Peer reviewed

Drosophila, *C. elegans*, zebrafish, and placental mammals. Analysis of genome sequences indicates that eta-tubulin is the last remaining tubulin family member to be characterized in vertebrates. Eta-tubulin is most similar to delta-tubulin, although it is clearly a distinct tubulin family. Eta-tubulin is also the least well-conserved of the tubulin families, both in terms of the number of eukaryotic lineages that have lost the gene and in the divergence of the protein within branches that have retained it. We have characterized *Xenopus laevis* eta-tubulin in a variety of cell lines and developmental stages. Eta-tubulin is part of a large cytoplasmic complex distinct from the gamma-tubulin ring complex, and does not localize with, or co-sediment with, microtubules. In multiciliated skin cells of the frog embryo, depletion of eta-tubulin results in abnormalities of basal body distribution, suggesting a role in cilia formation or function in vertebrates.

1962

Regulation of motor proteins by tubulin carboxy-terminal tails.

M. Sirajuddin[1], L. M. Rice[2], R. D. Vale[1]; [1]Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA, [2]Biophysics and Biochemistry, UT Southwestern, Dallas, TX

The cytoskeleton polymer, microtubules are composed of α/β tubulin heterodimers with a highly conserved structural core and a more variable unstructured, carboxy-terminal tail (CTT). Ever since the discovery of multiple α and β tubulin genes and tubulin posttranslational modifications (PTMs) it has been proposed that such variations might confer unique interactions with microtubule-associated proteins for specific cellular functions. This hypothesis, called 'tubulin-code' has received support from genetic studies, specific tissue or subcellular localizations of particular tubulin gene products or PTMs. However the inability to isolate distinct and homogenous tubulin species has hindered biochemical testing of this hypothesis. Since most of the variations, both genetic and PTMs occur in the CTTs, we engineered ~25 tubulin with distinct CTTs representing a single tubulin isotype or PTM. Using single molecule assays we tested the engineered microtubule interactions with four distinct classes of motor proteins (kinesin-1, -2, -13 and dynein). Our results show that tubulin isotype and PTMs can govern motor velocity, processivity and microtubule depolymerization rates. For some motors we observe that PTMs such as detyrosination and polyglutamylation is required for robust motility and using βIII tubulin we show that tubulin isotype specificity towards motors can be modulated by PTMs. Our results also reveal that different molecular motors recognize distinctive tubulin signatures supporting the premise of the tubulin-code hypothesis.

1963

β-tubulin carboxy-terminal tail regulates microtubule organization in the mitotic spindle

J. Aiken[1], C. Fees[1], J. Moore[1], E. T. O'Toole[2], T. H. Giddings Jr. [2]; [1]Department of Cell and Developmental Biology, University of Colorado School of Medicine, Aurora, CO, [2]Department of MCD Biology, University of Colorado at Boulder, Boulder, CO

Formation of the mitotic spindle requires coordination between microtubule assembly and the activities of microtubule motors and other binding proteins. Carboxy-terminal tail regions (CTTs) of α- and β-tubulins are likely to play an important role in this coordination. CTTs contain an abundance of negatively-charged residues that support electrostatic interactions at the microtubule surface, are major sites of tubulin posttranslational modification, and exhibit high sequence variation across species. We hypothesize that CTTs act as regulatory modules for tuning spindle organization and function. Here we examine the unique role of β-CTT. Using timelapse microscopy, we find that mutants lacking β-CTT are defective for spindle assembly and elongation during anaphase. Mutants lacking α-CTT do not exhibit these phenotypes. To understand the basis of spindle defects in β-CTT mutant cells, we examine the organization of spindle microtubules using 3-dimensional electron tomography. We find several striking defects. First, microtubules emanating from opposing spindle poles exhibit diminished interdigitation. This suggests a defect in forming or stabilizing antiparallel microtubule overlaps. We explore this by analyzing the localization and activity of proteins that are recruited to antiparallel overlaps. Second, mutant spindles contain microtubule fragments that are not anchored at the spindle poles. Our analyses indicate that these fragments are independent of γ-TuSC, and may therefore be formed by either spurious microtubule