although the p150^{Glued} subunit of the dynactin complex is up-regulated in BPAG1 null neurons, the dynein-dynactin motor complex remains intact. In the absence of BPAG1n4, however, the association between the motor-cargo complex and the microtubule track is drastically weakened. By yeast two-hybrid screen we found a novel neuron-specific membrane protein that interacts with BPAG1n4. Co-immunoprecipitation assays provide further evidence that BPAG1n4 interacts directly with the novel protein *in vivo*. Immunofluorescence staining reveals co-localization of BPAG1n4 and the novel protein on vesicles in sensory axons. We propose that BPAG1n4, anchored by the novel membrane protein to vesicular cargoes, functions to facilitate transport of dynein/dynactin-cargo supercomplexes by linking them to microtubules.

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Dynamics of Amyloplast Sedimentation in Bundle Sheath Cells of the Maize Pulvinus

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In stems of *Zea mays* L. changes in the gravity vector are perceived in the pulvinus, a disc-shaped tissue located above each node that responds to the gravity stimulus by differential growth resulting in upward bending of the stem. Gravity perception occurs in the bundle sheath cells, which contain starch-filled plastids (amyloplasts) that sediment to the base of the cells. It is hypothesized that interaction of amyloplasts with cytoskeletal elements as well as plasma membrane and/or internal membranes are important for transducing the mechanical stimulus into a physiological growth response. Using video microscopy and digital image analysis we recorded and tracked the path of amyloplasts in pulvinal sections over time, before and after gravistimulation and in the presence and absence of actin and microtubule disrupting drugs. In untreated pulvinal sections amyloplast saltations and particle movements can be observed in upright and gravistimulated tissue. Although the majority of amyloplasts settle at the bottom of the cells at about 15 minutes after turning, frequent upward movement against gravity occurs. In the presence of the actin depolymerizing drug latrunculin B (0.5 - 1 μM) amyloplasts tend to aggregate in clumps, and saltations and particle movements stop after 10-30 minutes. Upon rotation of the cells by 90° amyloplasts of latrunculin B treated cells appear to slide along the cell periphery as a group and either spread evenly at the cell base, or, after prolonged treatment, aggregate at the bottom corner of the cells. No upward movement occurs in these conditions. Treatment with the microtubule depolymerizing drug oryzalin has no discernable effect. The results support the hypothesis that interactions of amyloplasts with actin filaments have a role in early gravitropic signaling.

Centrosomes (2259-2278)

2259

The nucleus as a new microtubule organizing center in differentiating myoblasts

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During early stages of muscle fiber cell differentiation, myoblasts elongate and fuse into multinucleate myotubes. In the cytoplasm, this process is marked by the reorganization of the microtubule network. Whereas undifferentiated myoblasts possess microtubules that grow radially off the centrosome, microtubules in differentiating cells grow off the nuclear surface. Because the nucleus acts as a novel microtubule organizing center upon differentiation, we studied the reorganization of centrosome proteins in these cells. We discovered that a subset of centrosome proteins, including pericentrin, centrin-3, and PCM-1 re-localize to a rim around the cytoplasmic surface of the nuclear envelope. The re-localization of pericentrin depends on the presence of the protein PCM-1, but not vice-versa. Biochemical experiments indicate that PCM-1 is part of an insoluble matrix around the nucleus that is resistant to extraction with high salt or detergent and that requires high concentrations of denaturing reagents to solubilize. Heterologous cell fusion experiments of myoblasts with human U2Os cells indicate that the nuclear surface becomes competent for anchoring centrosome proteins and that the centrioles lose their pericentriolar material in a differentiating environment.

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Centrosomal attachment to the nucleus

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The centrosome and nucleus are intimately associated during interphase in a variety of organisms and cell types, yet the importance and mechanism of the attachment are just beginning to be understood. Mutations in the *C. elegans* gene *zyg-12* specifically perturb the attachment of the centrosome to the nucleus during early embryogenesis. Analysis of the mutant phenotypes demonstrates for the first time that this attachment is essential. *zyg-12* encodes multiple isoforms of Hook-like proteins. Hook proteins mediate the interaction of membrane-bound organelles such as the Golgi with the microtubule cytoskeleton. ZYG-12 isoforms localize to both nuclear envelope and centrosomes if they include a transmembrane domain or just centrosomes if they do not. ZYG-12 localization to the centrosomes, but not the nuclear envelope, requires microtubules. Conversely, ZYG-12 localization to the nuclear envelope, but not the centrosome, requires *sun-1,* a second gene required for centrosomal attachment. SUN-1 includes a SUN domain, which was originally identified in proteins that localize to the nuclear envelope. We are working to characterize both the mechanism of attachment and how ZYG-12 localizes to the nuclear envelope via a SUN-1 dependent mechanism.

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A Centrosomal Target of Human T-Cell Leukemia Virus Oncoprotein Tax

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Human T-cell leukemia virus type I (HTLV-I) is etiologically associated with adult T-cell leukemia/lymphoma. The Tax oncoprotein of HTLV-I is a multifunctional protein involved in transcriptional regulation, cell cycle control and leukemogenesis. Tax acts through interaction with various host factors. Previously we have identified novel binding partners for Tax, including human mitotic checkpoint protein MAD1 and IκB kinase regulatory subunit IKK-γ. In this study we report on the identification and characterization of another novel Tax-binding protein designated TXBP121. The protein-protein interaction between Tax and TXBP121 has been verified by GST pull-down assay, co-immunoprecipitation and co-localization studies. TXBP121 is a novel centrosomal protein with coiled-coil domains. TXBP121 tightly associates with γ-tubulin, pericentrin, and other centrosomal proteins through a C-terminal domain, which sufficiently targets the heterologous EGFP protein to the centrosome. TXBP121 can be phosphorylated both in vitro and in vivo by Cdk2/cyclin A, a key regulator of centrosomal functions. Interestingly, overexpression of TXBP121 in cultured cells inhibited centrosome duplication. TXBP121 forms a complex with Tax in vivo and it re-localizes a portion of Tax protein to the centrosome. Tax and loss of function mutants of TXBP121 induced centrosome hyper-amplification in HeLa and Jurkat cells. Co-expression of TXBP121 and Tax led to an inhibition of Tax-dependent centrosomal phenotypes. Our findings suggest a new model for viral transformation in which the Tax oncoprotein targets a centrosomal coiled coil protein leading to centrosome-associated genomic instability. This work was supported by grant HKU-7249/01M (to D.-Y. J.) from the Hong Kong Research Grants Council. D.-Y. J. is a Leukemia & Lymphoma Society Scholar.

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Roles of Nudel in Centrosome Assembly

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The centrosome serves as the microtubule-organizing center in most animal cells. It is composed of a pair of centrioles and a cloud of pericentriolar material (PCM). Proteins in PCM, including γ-tubulin, pericentrin, and PCM-1 often show dynamic associations with the centrosome during the cell cycle. Some, for instances, pericentrin and PCM-1, exhibit strong dependence on cytoplasmic dynein-mediated transport for their centrosome targeting, while the others, e.g., γ-tubulin, are less or even not dependent. Despite enormous studies, how the PCM is assembled at molecular level remains poorly understood. We have previously shown that Nudel, an evolutionarily conserved protein, regulates dynein motor activity in both membrane traffic and mitosis. Here, we show that it is also crucial for centrosome assembly. Silencing Nudel expression by RNA interference disrupted centrosomal pericentrin, dynein, and dynactin, and also induced dispersion of the PCM-1-containing granules, or the "centriolar satellites", in HeLa cells. Moreover, Nudel depletion also significantly attenuated centrosomal γ-tubulin, suggesting a role to recruit γ-tubulin. Like γ-tubulin, Nudel exhibited dynamic centrosomal localization with rapid turnover rates independent of dynein-mediated transport, further suggesting it as a central centrosome component. In addition, both the microtubule nucleation and anchoring were suppressed in Nudel-depleted cells. While the former phenotype was attributed to insufficient centrosomal γ-tubulin, the latter was probably due to loss of dynactin, another dynein regulator critical for microtubule anchoring at centrosomes. We also provided evidence showing that the heterotrimeric G protein subunit β plays important roles in centrosome assembly through direct interaction with Nudel.

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BRCA1 regulates centrosome function by inhibiting microtubule nucleation activity

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BRCA1 is a breast and ovarian-specific tumor suppressor, which has multiple functions in normal cells. It is a multi-functional nuclear phosphoprotein with most functions aimed to maintain the stability of the genome. These include its role in transcription control, cell cycle regulation, chromatin remodeling, and DNA repair. BRCA1 has a RING domain at the N-terminus that is important for its function as an E3 ubiquitin ligase, when associated with the BARD1 protein. BRCA1 localizes to centrosomes. Data from our lab demonstrate that BRCA1-dependent ubiquitination activity inhibits centrosome duplication. In this study