In Brief

Microtubule Interactions with Regulatory Proteins

A New Microtubule-associated Protein in Drosophila

By harnessing the power of fruit fly genetics, Cullen et al. (page 1005) have isolated a new microtubule-associated protein involved in mitotic spindle formation. This finding also demonstrates the feasibility of using *Drosophila* genetics to study microtubule-associated proteins (MAPs) in vivo in higher eukaryotes.

MAPs have been studied extensively in vitro, but genetic studies on these proteins in living cells have been done primarily in yeast, whose mitotic machinery differs from that of higher eukaryotes. In this new work, the researchers screened fly mutants for defects in mitosis, and isolated a mutant, subsequently named *mini spindles* (*msps*), in which the integrity of mitotic spindles is disrupted. Molecular cloning demonstrated that the mutated gene encodes a MAP that is conserved among eukaryotes. The Msps protein localizes to mitotic spindles and centrosomal regions in a cell cycle-dependent manner. The findings are consistent with a model where Msps is involved either in microtubule bundling or in the formation of long microtubules. "In any case a better understanding of spindle defects in mutants and the Msps protein itself is crucial," says corresponding author Hiroyuki Ohkura, who adds that the group is already taking advantage of the system's genetics to dissect the mechanism.

Microtubule Targeting to Substrate Contacts

Starting on page 1033, Kaverina et al. report using video microscopy to show that microtubules repeatedly target substrate contact sites in migrating cells and link the frequency of microtubule targeting to the turnover of contact sites. These findings suggest a mechanism by which microtubules may exert control over cell polarity.

Previous work has shown that microtubules are involved in regulating the size of focal adhesions, but the regulatory mechanism remains unknown. Using fluorescently tagged proteins and video imaging, Kaverina et al. determined that contact turnover in spreading and moving cells is retarded in the absence of microtubules, and that the retraction of cell edges during cell motility follows the repetitive targeting of peripheral substrate contacts. In motile cells, the frequency of microtubule targeting is eightfold lower for contact sites on the leading edge of the cell than for the smaller contact sites on the trailing edge. These results are consistent with a model in which microtubules deliver localized doses of relaxation signals to the sites. While the team is now working to determine the specific signals governing substrate contact sites, J. Victor Small, corresponding author, suggests that microtubules may have a similar role in other cellular processes: "We think that microtubule motors might indeed be transporting other signals, either by reversibly binding protein complexes or through the local delivery of vesicles bearing signaling molecules."

Overturning a Model for the Timing of Cytokinesis

Using echinoderm eggs as a model, Shuster and Burgess (page 981) have found that the timing of cytokinesis does not appear to be controlled by inhibitory phosphorylation of the myosin II light chain. These results are inconsistent with a popular model of this process, which was based on earlier in vitro data.

Biochemical studies have shown that the cyclin-dependent kinase $p34^{cdc2}$ can phosphorylate the myosin light chain protein LC20 on two residues, inhibiting myosin activity. Because $p34^{cdc2}$ activity declines during anaphase, the in vitro data supported a model in which a decrease in inhibitory phosphorylation led to an increase in myosin activity and the start of cytokinesis.

In this new work, radioactive labeling of echinoderm eggs undergoing mitosis demonstrates that despite high levels of $p34^{cdc2}$ activity, LC20 from cortical myosin is not phosphorylated on the inhibitory sites at any time during mitosis, but is phosphorylated on an activating site during anaphase and telophase. The authors also show that even in the presence of elevated $p34^{cdc2}$ activity, a contractile ring can form in response to metaphase asters. The results suggest that, while $p34^{cdc2}$ may have a role in regulating the timing of cytokinesis, it does not act by directly inhibiting myosin II activity. This finding is further bolstered by recent genetic evidence from *Schizosaccharomyces pombe*, in which mutations in the phosphorylation sites of the myosin light chain had no effect on the timing of cytokinesis (McCollum, D., A. Feoktistova, and K.L. Gould. 1999. *J. Biol. Chem*. 274:17691–17695).

Role of Actin in Maintaining Spindle Orientation

In reexamining the role of actin in yeast mitotic spindle orientation, Theesfeld et al. (page 1019) discovered that, contrary to previous reports, actin is required only in establishing spindle orientation, not in maintaining it. When *Saccharomyces cerevisiae* undergoes mitosis, the spindle must align near the neck of the newly emerging bud, a process that seems to require cytoplasmic microtubules. Earlier studies on actin mutants mitotically arrested with hydroxyurea led to a widely embraced model in which actin is important for maintaining proper spindle orientation throughout mitosis.

The new work relies on pharmacological inhibition of F-actin and a reanalysis of the mutants, and the results suggest that F-actin has a role in establishing spindle orientation, but is not required for the maintenance of orientation late in the cell cycle. While the mechanism of spindle orientation appears to change in the course of the cell cycle, other details of the process remain unclear. "A popular option is that actin delivers microtubule capture-sites to the bud, and I guess in the simplest case one could imagine that after some vague 'maturation,' such sites

could then remain asymmetrically distributed even without actin," says corresponding author Daniel Lew.

The authors suggest that the earlier data may have been skewed by the inclusion of cells that were not completely arrested by the hydroxyurea treatment.

Synergistic Signals for Neuronal Survival

Through biochemical analysis of rat sympathetic neurons, Vaillant et al. (page 955) have mapped the convergence of two signals that act synergistically to promote neuronal survival. In addition to illuminating an important process in brain development, the work may aid efforts to develop new therapies for damaged neurons.

Earlier data demonstrated that growth factors and neuronal activity coordinately regulate neuronal survival, but the convergence of the two signaling pathways had not been mapped. Vaillant et al. found that when neurons are exposed to suboptimal levels of the neurotrophin NGF, depolarization with levels of KCl that have no survival effect on their own synergistically increase survival. Since neurons in the developing brain compete for neurotrophins, the synergistic effect of depolarization should confer an in vivo survival advantage on active neurons. Biochemical and function-blocking experiments show that the two signals converge on the intracellular Ras-PI3-kinase-Akt pathway. Corresponding author Freda Miller adds that "This certainly is not limited to mammals, since it is clear that in probably all vertebrates, there is an interplay between growth factors and activity that regulates neuronal survival." The team is now pursuing similar studies in sensory and motor neurons.

By Alan W. Dove, 712 W. 176th St., New York, NY 10033. E-mail: a.dove@erols.com