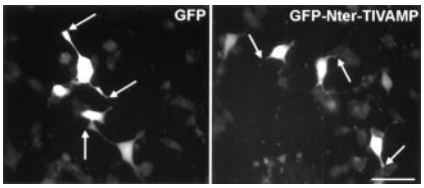
Mechanisms of Vesicular Transport in Neurite Outgrowth

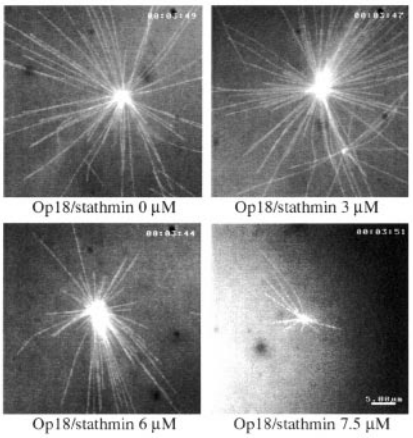
Beginning on page 889, Martinez-Arca et al. describe the role of the tetanus neurotoxin insensitive vesicle-associated membrane protein (TI-VAMP) in neurite outgrowth. Through a combination of biochemical and functional analyses, the authors show that TI-VAMP is required for vesicular transport in neurite outgrowth, and identify the NH_2-terminal domain of the protein as a major regulator of this process. In addition to providing significant new insight into vesicular trafficking, the findings suggest that TI-VAMP could be a valuable pharmacological target in efforts to treat nerve damage.



Though neurite outgrowth is a critical event in neuronal differentiation, the vesicle-targeting mechanisms driving the process have remained poorly understood. In this new work, the authors show that TI-VAMP is essential for neurite outgrowth in PC12 cells, and that the NH_2-terminal domain of the protein inhibits TI-VAMP association with the synaptosome-associated protein SNAP25. Expression of the NH_2-terminal domain of TI-VAMP in PC12 cells strongly inhibits neurite outgrowth, and expression of TI-VAMP lacking the NH_2-terminal domain stimulates neurite outgrowth. Because TI-VAMP localizes to the axonal and dendritic growth cones of hippocampal neurons in primary culture, the same mechanism is likely to be at work in vivo. The authors are now hoping to identify the proteins and lipids transported in TI-VAMP–containing vesicles, factors that are likely to be involved in neurite elongation.

Structural Transitions at Microtubule Ends

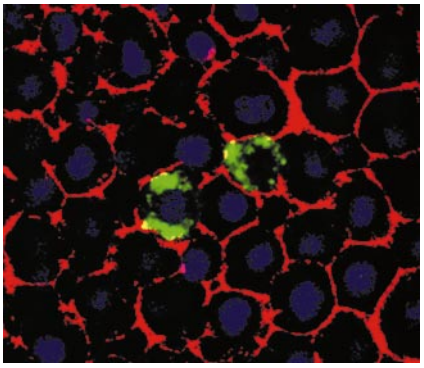
In the first detailed study of microtubule end structure and dynamics performed under physiological conditions, Arnal et al. (page 767) have found that microtubule assembly involves the extension of a two-dimensional sheet of protofilaments which then closes into a tube. In addition to demonstrating the feasibility of studying microtubule end structure in a physiologically relevant system, the results support a model that helps to explain dynamic instability.



Earlier studies using electron microscopy and pure tubulin demonstrated that microtubule assembly is regulated by changes in microtubule end structure, but the biochemical details of this process and its relevance to in vivo tubulin behavior remained unclear. In this new work, the authors examined microtubules in *Xenopus* egg extracts by electron cryomicroscopy and used the catastrophe-promoting factor Op18/stathmin to modulate microtubule dynamic instability. The results show that microtubule assembly involves the growth of two-dimensional sheets of tubulin, which later close into a tube. Increasing the concentration of Op18/stathmin causes a decrease in the length and prevalence of the sheets at microtubule ends and an increase in blunt and frayed ends. The findings suggest that microtubules shrink by losing protofilaments from frayed ends, and that blunt-ended microtubules might represent a metastable intermediate between the growing and shrinking states. The ability to correlate growing and shrinking microtubules with their end structures should facilitate further studies on the regulation of microtubule dynamics.

Germ Plasm Segregation and Specification

By studying the segregation of *vasa* gene products, which encode an RNA helicase that marks the germline in a variety of organisms, Knaut et al. (page 875) have obtained strong supporting data for a new model of germ cell specification in zebrafish. The authors propose that *vasa* RNA, but not its protein, is a component of the zebrafish germ plasm, and that maternal signals trigger the pattern of germ plasm segregation leading to germline fate commitment. The new model, combined with the well-defined genetics and transparency of the zebrafish system, should facilitate future studies on this crucial developmental process.



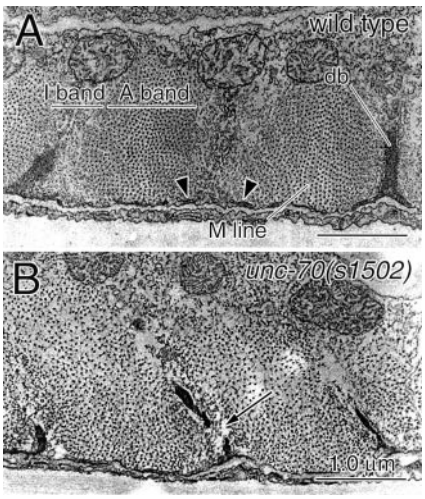
Though germ cell specification and *vasa* localization have been correlated in *Xenopus*, *Drosophila*, and other model systems, both phenomena remained poorly understood in fish. In this new work, the researchers found that *vasa* RNA, but not Vasa protein, localizes to a subcellular structure as-

sociated with germ plasm. The RNA segregates asymmetrically during cell division until the late blastula stage, when *vasa* RNA segregation becomes symmetric in the founder population of primordial germ cells. In embryos carrying a mutation in the maternal effect gene *nebel*, asymmetric segregation of *vasa* RNA is impaired. Based on these results, the authors propose that unequal germ plasm segregation establishes a separate population of four cells with the potential to form the germline. A maternal program induces these cells to become the founder population of the germline, and germ plasm is segregated symmetrically in subsequent cell divisions.

Functions of Spectrin in C. elegans

Using different approaches, Moorthy et al. (page 915) and Hammarlund et al. (page 931) have analyzed the roles of spectrin in the biology of *C. elegans*. The mutually reinforcing results overturn some earlier hypotheses about the functions of the β-G spectrin subunit, and define a variety of specific roles for spectrin subunits in both developing and adult worms.

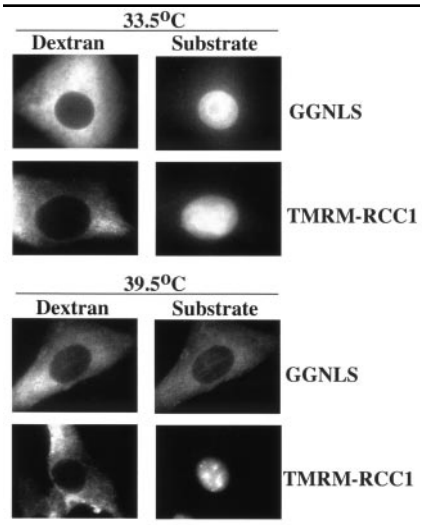
A major component of the membrane skeleton in most metazoans, β-spectrin has been proposed as a factor in membrane stabilization, the localization of specific membrane proteins, and the generation of cell polarity. Hammarlund et al. found that the *unc-70* gene encodes the *C. elegans* homologue of β-G spectrin, and determined growth conditions that allow the survival of *unc-70* null worms, a mutation previously believed to be lethal. Contradicting previously proposed roles for β-G spectrin, membrane integrity and cell polarity are not impaired in the *unc-70* null mutants, but axon outgrowth and muscle organization are affected.



Moorthy et al. performed a global analysis of the three spectrins encoded by the *C. elegans* genome: the β-G spectrin studied by the Hammarlund team, β-H spectrin, and α-spectrin. Using *RNAi* to inhibit expression, the authors found that the phenotype caused by a loss of α-spectrin is reproduced by inhibiting both β-G spectrin and β-H spectrin. This result, combined with global expression profiles of the three spectrin subunits, support a model in which α-spectrin combines with β-G and β-H subunits in different tissues to carry out the diverse functions of spectrin. In addition, the *RNAi* experiments confirm that β-G spectrin is not required for establishing cell polarity.

Nuclear Import of RCC1

Nemergut and Macara (page 835) studied the nuclear import of RCC1, the guanine-nucleotide exchange factor for the Ran GTPase, and found that RCC1 import into the nucleus can proceed by at least two distinct mechanisms. The results help explain a puzzling problem: since enrichment of RCC1 in the nucleus is believed to be a requirement for the nuclear import activity of Ran, it was unclear how Ran-dependent mechanisms could account for the initial establishment of a nuclear pool of RCC1.



By time-lapse photography, the authors found that RCC1 import into the nucleus is one of the most rapid nuclear import processes yet described. When the NH_2-terminal domain of RCC1, containing a NLS, is deleted, the protein is still imported into the nucleus. In addition to the classical nuclear import pathway, RCC1 can use a pathway that is independent of the NLS, importin-α binding, and Ran. The second pathway is saturable, but does not require energy. Based on their results, and the central role of RCC1 in nuclear import, the authors propose that the second import pathway evolved to scavenge free RCC1 from the cytoplasm and ensure that the protein is enriched in the nucleus.

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