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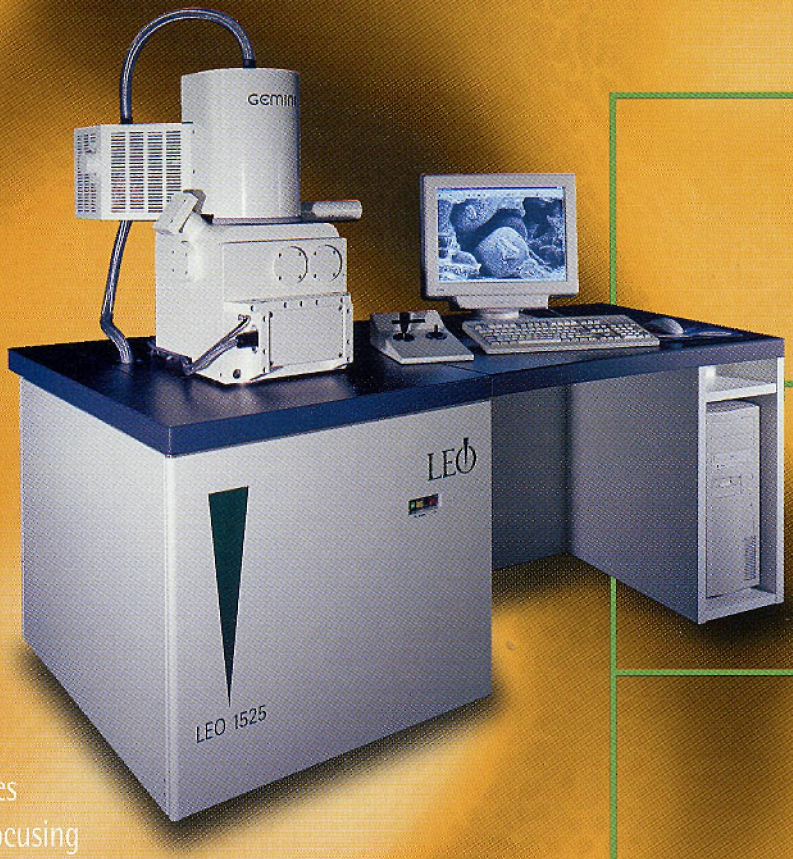

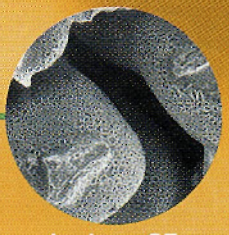

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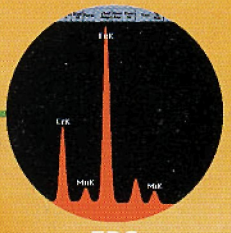

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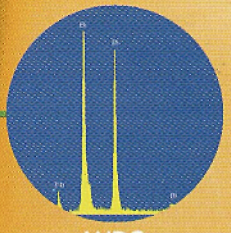

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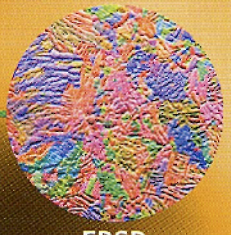

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WATCHING RAFTS MOVE WITHIN CELLS: A FLUORESCENCE MICROSCOPE-BASED TRANSPORT ASSAY

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Mayo Clinic

Imagine a raft in a canal between point A and point B. On that raft is a visible (fluorescent) cargo. Also, attached to that raft is a motor that will propel the raft only from A to B (anterograde transport). When the raft gets to point B, another motor is attached that can propel the raft, and its cargo, and the anterograde motor, back to point A (retrograde transport). Within a cell, the canals are microtubules, and a lot is known about anterograde and retrograde transport in some systems, but these phenomena have not been directly observed in a living, intact animal. Until now, that is. In a pair of very interesting papers, the laboratory of Jonathan Scholey has shown us convincing micrographs of anterograde[2] and retrograde[3] transport in an important animal model.

Using the nematode Caenorhabditis elegans, Scholey's group introduced chimeric genes encoding a fusion of Green Fluorescent Protein (GFP) and several specific target proteins. Using a fluorescence microscope, they could visualize fluorescing dots that moved within the animal and measured the speed of the movement. Specifically, they looked at intraflagellar transport (IFT) in cilia of chemosensory cells that function as the "nose" of this worm. One of the proteins they studied, referred to as OSM-6, is a component of a macromolecular complex that is actually called an "IFT raft." Using the fluorescent GFP as a marker for the location of OSM-6, they could visualize and quantitate the movement of the IFT raft from the base to the tip of a cilium. In addition, they linked GFP to a subunit of a member of the kinesin family, molecules known to drive anterograde transport. Interestingly, they found that the raft (OSM-6), and its motor (the kinesin subunit), traveled at the identical speed of 0.65 µm per second. To bring these movements into macroscopic virtual perspective by scaling up from a microtubule with a diameter of 24 nm to a canal 1 meter wide, the virtual raft is moving along at about 94 km/hour (about 63 miles per hour). That's a fast moving raft with a powerful motor!

Concerned that the recording technique could give the same speed of travel for all proteins measured, Scholey's group observed the movement a ciliary transmembrane receptor (referred to as ODR-10) with GFP and measured its speed at 1.59 µm per second (blazing along at 153 mph in our scaled-up scenario). This established that the identical speeds recorded for the raft and the motor were not an artifact of the recording technique, but rather they were traveling together.

In the second paper, Signor *et al.* measured retrograde (from the tip of the cilium back to the base) transport of the molecules thought to be components of the IFT raft (called OSM-1 and OSM-6) and for the kinesin molecule (the anterograde motor that would accumulate at the tip of the cilium if not transported back). The speed of this return trip was about 1.1 µm per second (over 100 mph!). But it was known that a different molecular motor drives retrograde transport. To dissect out this part of the mechanism, Scholey's group worked with a mutant of *C. elegans* that lacked a specific molecule of the dynein family (referred to as the class DHC1b cytoplasmic dynein CHE-3). In the animals lacking this particular molecule, anterograde transport of the IFT raft was normal, but retrograde transport was inhibited, implicating this molecule as the specific molecular motor for retrograde transport of the raft, along with its cargo, including the anterograde motor.

These papers present a novel biologic assay for intracellular transport in an intact animal. In addition, they demonstrate the usefulness of the C. *elegans* model for studying transport. Genetic studies have identified 25 genes that are essential for ciliary function in this animal. Scholey's group have shown that specific gene products can be tagged (in this case, with GFP), or deleted, and the role of the gene product on ciliary function demonstrated. Since cilia (and the structurally similar flagella) are important biologic structures, this paves the way for even more interesting studies. ■

1 The authors gratefully acknowledge Dr. Jonathan Scholey for reviewing this article.

2 Orozco, J.T., K.P. Wedaman, D. Signor, H. Brown, L. Rose, and J.M. Scholey, Movement of motor and cargo along cilia, Nature 398:674, 1999.

3 Signor, D., K.P. Wedaman, J.T. Orozco, N.D. Dwyer, C.I. Bargmann, L.S. Rose, and J.M. Scholey, Role of class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*, J. Cell Biol. 147:519-530, 1999. Video images from this study can be accessed at http://www.mcb.ucdavis.edu/faculty-labs/scholey/

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