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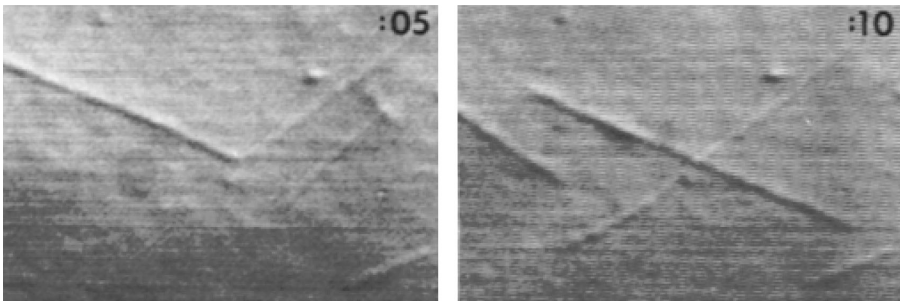
MAP 1C is a motor

When the protein dynein was discovered to provide the flagellar bending force to axonemal microtubules (MTs) (Gibbons, 1963), "people had jumped to the reasonable hypothesis that dynein was perhaps also involved in other microtubule movements," recalls Richard Vallee (Columbia University Medical Center, New York, NY). But by the 1980s, no such cytoplasmic motor proteins had been found.



MAP 1C, now known as dynein, translocates microtubules.

Kinesin and MAP 1C

In the summer of 1985, Vallee recalls seeing work on axonal transport in the squid axoplasm from the labs of Michael Sheetz and Ray Lasek at the Woods Hole Marine Biology Laboratory. "The thinking about axonal transport was all over the place before that," he says. Observations of fast axonal transport argued against a passive mechanism, but no one had found a mechanism to support theories like cytoplasmic streaming along the MTs. "But now," says Vallee, "there was good evidence that there might be specific molecules responsible for transport." The Sheetz lab identified and named the new molecule kinesin and showed it could move both MTs on glass and axonal organelles along MTs (Vale et al., 1985a).

At about this time, Bryce Paschal joined Vallee's lab at The Worcester Foundation for Experimental Biology (Shrewsbury, MA) as a graduate student. A few months into his Ph.D., Paschal had second thoughts about graduate school and took a leave of absence, but he continued working with Vallee as a technician. He began working on a project to test whether kinesin had an MT-stimulated ATPase activity. He purified kinesin, tested column fractions for ATPase activity, and noticed that he got two peaks of activity—one that tracked to kinesin, but another in fractions containing MT-associated protein 1C (MAP 1C), previously identified in the lab. Another lab reported that kinesin's activity was dependent on MTs (Kuznetsov and Gelfand, 1986), so Paschal turned his attention to MAP 1C.

If it looks like a motor...

Trace MAP 1C had always been found in the lab preparations of calf brain MTs. Preliminary characterization had shown that MAP 1C was insensitive to proteolysis, unlike other MAPs (Bloom et al., 1984). In 1982 Vallee had developed a new protocol for MT preparations using taxol, which was such a potent promoter of MT assembly that preps could be made in the absence of ATP or GTP nucleotide (Vallee, 1982).

In this situation, MAP 1C became much more abundant. Nucleotide-sensitive MT association "was very characteristic of a motor protein," says Vallee. In previous preps using nucleotide, he realized, "all of us had been throwing milligrams and milligrams of motor proteins down the drain. It explained why these proteins had not popped up before."

Thinking they were on the trail of the elusive cytoplasmic dynein, Vallee wanted more definitive proof. The best test was to do scanning transmission EM on the protein itself and compare its structure directly to that of flagellar dynein. The first images, produced early in the project, were conclusive. "There was no question that this thing was dynein," says Vallee. Paschal set to work to show that MAP 1C acted as an MT motor. He took on the tricky MTs-on-glass motility assay and demonstrated that, in a kinesin-free prep, MAP 1C could translocate MTs in a unidirectional manner (Paschal et al., 1987a).

Anterograde transport by kinesin had been demonstrated (Vale et al., 1985b). "There was no indication that kinesin could mediate bidirectional transport, but decades of neurobiology had established the retrograde movement of proteins," says Paschal, now at the University of Virginia (Charlottesville, VA). Using *Chlamydomonas reinhardtii* flagella that have a defined polarity, he showed that MAP 1C and kinesin moved the axonemes in opposite directions (Paschal and Vallee, 1987), and that MAP 1C was the retrograde motor.

The clincher was the publication of EM pictures showing that MAP 1C was in fact a two-headed cytoplasmic dynein (Vallee et al., 1988). Paschal went on to show that flagellar dynein isolated from sea urchin sperm behaved similarly in his MT motility assays (Paschal et al., 1987b). In all, it was a banner year for him, with four major publications that largely solved the vexing question of how cells moved things along MTs in two distinct directions. It was definitely worth the 5 a.m. drives to Cambridge, MA to pick up calf brains from a slaughterhouse and, "needless to say," says Paschal, "I decided to go back to graduate school." JCB

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