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The discovery of tubulin

In 1963, improved fixation methods led to the definitive identification of microtubules (see “Microtubules get a name” *JCB*. 168:852). Just one year later, Gary Borisy embarked on a daring project to isolate the main component of those microtubules.

The effort was initiated by Edwin Taylor at the University of Chicago. Taylor was interested in using colchicine to study mitosis. Unfortunately, “the literature on the effects of colchicine was very confused,” says Borisy. Colchicine was known to destroy the mitotic spindle but could also inhibit a disparate collection of other processes including distracting oddi-

Further correlation came when the colchicine-binding activity was extracted under low salt conditions that led to the disappearance of microtubules (Borisy and Taylor, 1967b). The group took pains to measure detailed in vitro binding kinetics and show that they matched those seen for intact cells, where colchicine disassembled microtubules. “The results,” concluded the authors, “are consistent with the hypothesis that the binding site is the subunit protein of microtubules.”

For now, the protein was nameless. “We did not give it a name, which was a

Harvard was probably looking at a monomeric version of what Taylor’s group was isolating under less-denaturing conditions (Gibbons, 1963; Renaud et al., 1968).

For Gibbons, cilia had the advantage of having a vast excess of tubulin over other proteins, although early on it was far from certain that the same main component would be the basis of “microtubules” from flagella, cilia, the cytoplasm, and spindles. Not only did this turn out to be the case, but the Gibbons group also

spotted that the microtubules consisted of two closely related proteins (Renaud et al., 1968).

By 1966, the Taylor group had a protein that was pure to homogeneity, and Borisy left for his postdoc as the characterization of tubulin continued. “I was extremely lucky to have had the chance to work in Ed’s lab,” he says. Going after tubulin “was a really gutsy thing to do as an assistant professor.” But Taylor, and certainly Borisy (now at Northwestern University, Chicago, IL), had no regrets. After some worrying and confusing times, “things worked out.” **WW**

Tubulin is isolated by Gary Borisy and Edwin Taylor as a colchicine-binding activity, and by Ian Gibbon’s group from cilia.

ties such as cellulose deposition in plants.

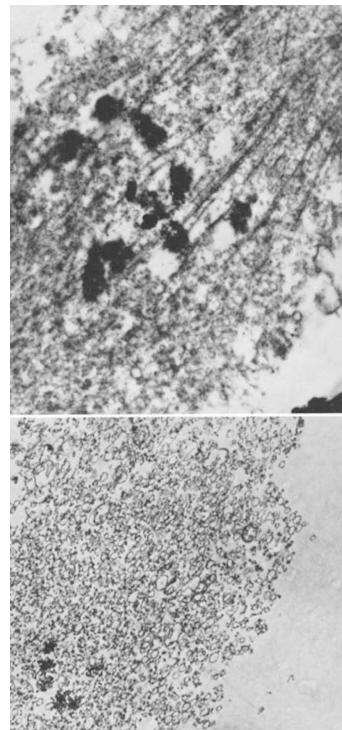
Colchicine did, however, bind with high affinity and simple kinetics to cells, suggesting that isolation of a complex of colchicine with its binding protein(s) should be possible (Taylor, 1965). As a new graduate student in Taylor’s lab, Borisy “got very excited” by the prospect of finding the colchicine-binding protein. “I begged to do this as a thesis project,” says Borisy. “He said no, no, no, it’s too risky. But I begged to do it.” Others told him, “‘You’ll have nonspecific binding and it’ll be a mess.’ But what did I know—I was a student.”

Colchicine did turn out to be specific, and Borisy did succeed in isolating a colchicine-binding activity from extracts of tissue culture cells (Borisy and Taylor, 1967a). The highest binding activity came from dividing cells, the isolated mitotic apparatus (Borisy and Taylor, 1967b), cilia, sperm tails (Shelanski and Taylor, 1967), and brain tissue. The brain tissue was a temporary scare: “It seemed like, oh my goodness, this is a totally nonspecific binding reaction and it’s a mess,” says Borisy. But there was a common denominator in that all the sources had an abundance of microtubules.

blunder,” says Borisy. Although tubulin was the obvious candidate given the existing name of microtubules, “it sounded so jarring to our ears.”

But soon enough Mohri (1968) “gave it the obvious name—the name we considered and rejected.” The term “tubulin” was now official, although “spactin,” “flactin,” and “tektin” stuck around as alternative monikers for a little while (Satir, 1968).

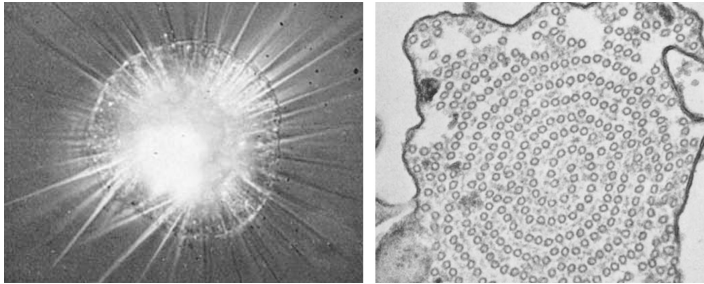
As purification from sperm tails (Shelanski and Taylor, 1968) and then brain (Weisenberg et al., 1968) continued, “there were many red herring findings and inconsistent findings and blind alleys,” says Borisy. Initially there were candidate microtubule proteins of very different sizes from both Daniel Mazia’s study of the mitotic apparatus and Ian Gibbon’s study of *Tetrahymena* cilia. The Mazia candidate turned out to be a yolk protein contaminant, and the Gibbons group at



Conditions that deplete colchicine-binding activity (bottom) cause microtubules to disappear.

Borisy, G.G., and E.W. Taylor. 1967a. *J. Cell Biol.* 34:525–533.
Borisy, G.G., and E.W. Taylor. 1967b. *J. Cell Biol.* 34:535–548.
Gibbons, I.R. 1963. *Proc. Natl. Acad. Sci. USA.* 50:1002–1010.
Mohri, H. 1968. *Nature.* 217:1053–1054.
Renaud, F.L., et al. 1968. *J. Cell Biol.* 36:79–90.
Satir, P. 1968. *J. Cell Biol.* 39:77–94.
Shelanski, M.L., and E.W. Taylor. 1967. *J. Cell Biol.* 34:549–554.
Shelanski, M.L., and E.W. Taylor. 1968. *J. Cell Biol.* 38:304–315.
Taylor, E.W. 1965. *J. Cell Biol.* 25:145–160.
Weisenberg, R.C., et al. 1968. *Biochemistry.* 7:4466–4479.

Microtubules shape the cell



Axopodial spikes (left) owe their shape to an array of microtubules (right).

Soon after microtubules were first described by electron microscopy, several investigators began suggesting that they were structural elements (Byers and Porter, 1964) because they were localized to sites where cells were changing their shape. In 1967, Lewis Tilney and Keith Porter, then at Harvard University, provided direct experimental evidence that microtubule polymerization was important for the development and maintenance of cell shape.

Earlier work by Inoué (1952) had shown that when cells are exposed to cold temperatures the mitotic spindle—later shown to be composed of microtubules—disappears. Working with the protozoan *Actinosphaerium nucleofilum*, which has needle-like extensions (axopodia) consisting of a well-defined system of microtubules, Tilney and Porter reasoned that “if the microtubules are instrumental in the maintenance of these slender protoplasmic extensions, then low temperature, which, as previously stated, should cause the breakdown of the microtubules, ought secondarily to cause retraction of the axopodia.”

Their results supported this hypothesis. Cold treatment of *A. nucleofilum* cells caused the microtubules to disassemble and the axopodia to withdraw; after returning the cells to room temperature for a few minutes, the microtubules started to reassemble and the axopodia reformed (Tilney and Porter, 1967). The authors concluded that “microtubules are intimately involved not only with the maintenance of the axopodia but also with their growth.” Experiments performed at about the same time showed that treatment of *A. nucleofilum* cells with agents that cause microtubule depolymerization—mainly, hydrostatic pressure and colchicine treatment—gave similar results (Tilney et al., 1966; Tilney, 1968). Importantly, later work by Tilney and Gibbins (1969) established that microtubules also help change cell structure in higher organisms. The authors treated embryos of the purple sea urchin *Arbacia punctulata* with colchicine and hydrostatic pressure at different stages of development. Disassembly of the microtubules with these treatments prevented the characteristic cell shape changes in, and thus differentiation of, the mesenchyme of the developing embryo. **LB**

Byers, B., and K.R. Porter. 1964. *Proc. Natl. Acad. Sci. USA*. 52:1091–1099.
Inoué, S. 1952. *Biol. Bull.* 103:316.

Tilney, L.G., and K.R. Porter. 1967. *J. Cell Biol.* 34:327–343.

Tilney, L.G. 1968. *J. Cell Sci.* 3:549–562.

Tilney, L.G., Y. Hiramoto, and D. Marsland. 1966. *J. Cell Biol.* 29:77–95.

Tilney, L.G., and J.R. Gibbins. 1969. *J. Cell Biol.* 41:227–250.

Since the small vesicles, which Farquhar refers to as “Golgi vesicles,” carry lytic enzymes, the authors concluded they correspond to primary lysosomes—particles that contain hydrolytic enzymes but have not yet participated in digestion. Today it is known that these so-called Golgi vesicles belong to the family of clathrin-coated vesicles. **LB**

How to make a lysosome

Coated vesicles had been described as early as 1961. These small membranous structures were characterized by a highly organized layer of material on the cytoplasmic surface, “but no one knew their function,” says Marilyn Farquhar, whose lab at the time had become interested in how the Golgi complex helped produce enzymes.

Roth and Porter (1964) had provided evidence that coated vesicles have specialized functions in the cellular uptake of proteins. But for Friend and Farquhar (1967), the simple idea of uptake was not enough. They helped cement the idea that cellular trafficking involved an intersecting set of cellular highways.

They demonstrated that cells contain different types of coated vesicles, and that these vesicles are not only involved in protein uptake but also in the transport of lysosomal enzymes. The two pathways converged in multivesicular bodies (now known as endosomes), explaining how proteins could be endocytosed and then processed by cellular enzymes. The processing, we now know, takes place only after endosomes have either matured into or sent vesicles to lysosomes.



Vesicles large (lv) and small (sv) deliver contents to the incipient lysosome.

The 1967 paper “was quite unique for its time,” says Farquhar, because the group used three labels: soluble horseradish peroxidase as a tracer for protein uptake; thiamine pyrophosphatase as a Golgi marker; and acid phosphatase as a lysosomal enzyme marker. Using this method her group was able to distinguish among different types of vesicles and follow their direction of movement during protein uptake.

One type of coated vesicle, which was larger in diameter, formed at the cell surface by pinocytotic invagination of the apical cell membrane. It moved toward and fused with multivesicular bodies, thereby serving to transport endocytosed protein inside the cell. The other type of coated vesicle, smaller in size, seemed to originate from the Golgi cisternae and serve, at least in part, to transport the enzyme acid phosphatase and possibly other acid hydrolases from their site of packaging in the Golgi to multivesicular bodies, thus uniting the endocytosed and lytic proteins.

Bainton, D.F., and M.G. Farquhar. 1968. *J. Cell Biol.* 39:286–298.
Bainton, D.F., and M.G. Farquhar. 1968. *J. Cell Biol.* 39:299–317.
Friend, D.S., and M.G. Farquhar. 1967. *J. Cell Biol.* 35:357–376.
Roth, T.F., and K.R. Porter. 1964. *J. Cell Biol.* 20:313–332.