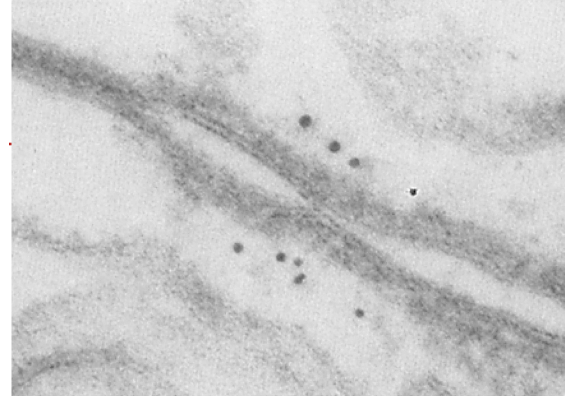
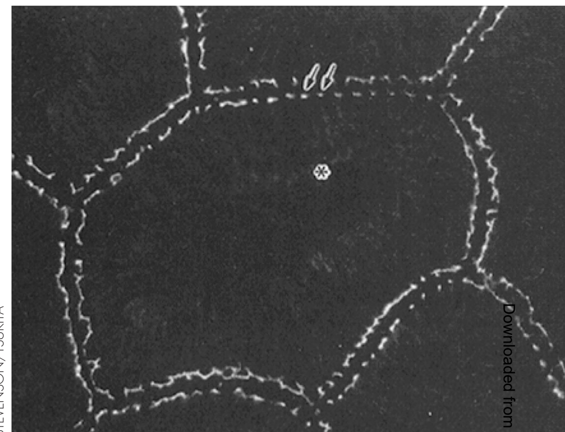
From the Archive

Sticking it out with tight junctions





STEVENSON/TSUKITA

ZO-1 (top) and occludin (bottom) both localize to tight junctions.

If patience is a virtue, then Daniel Goodenough and Bruce Stevenson earned their wings in the pursuit of the first tight junction protein. Stevenson spent seven years as a graduate student with Goodenough (Harvard Medical School, Boston, MA) before the two worked out the conditions to purify fragile tight junction–enriched preparations from mouse liver (Stevenson and Goodenough, 1984).

It was enough to jump-start the field of tight junction biochemistry, some 20 years after the first morphological description of these junctions, which give epithelial cells their ability to seal off body compartments (Farquhar and Palade, 1963). The preparations yielded multiple protein bands—two major and six minor.

From band to protein

Stevenson took the project with him to Mark Mooseker's lab at Yale University (New Haven, CT) as a postdoc and collaborated with Janet Siliciano in the Goodenough lab. Using the tight junction fraction, they screened monoclonal antibodies by looking for localization to the junctions by immunofluorescence EM. Commuting back up to Boston in his Ford Fiesta, Stevenson and Siliciano worked on the tedious process of antibody production. At first, some of their hybridomas would turn up positive, only to be lost upon cloning out to a 96-well plate.

With persistence and a species change, tight junction proteins are isolated.

On a Saturday before heading out to the beach, Stevenson says he remembers checking two plates, and being prepared to throw them out, only to find all of the 48 wells turning up positive. He cancelled his vacation and set to cloning the colonies out for the rest of the day. His perseverance led to the antibody that identified the first tight junction protein, ZO-1 (Stevenson et al., 1986). The protein occurred in many other epithelial cell tight junctions including those of rat liver and mouse colon, kidney, and testis. The widespread distribution of the protein, cloned a few years later (Anderson et al., 1989), argued that it could be a "ubiquitous component of all mammalian tight junctions."

More than ZO-1

But it was clear early on that, as a peripheral membrane protein, ZO-1 was not the integral junction-forming protein. Finding this protein was still up for grabs, and those in the United States went back to their tight junction preps to try to generate other antibodies. But contamination with the very antigenic ZO-1 and gap junction proteins foiled those efforts.

In the end, the prize went to Shoichiro Tsukita's group, then at the National Institute for Physiological Sciences in Okazaki, Japan. "Tsukita," says Goodenough, "had a wonderful insight" in switching species and using chicken as the source of antigen.

Tsukita recalls that by 1985 the problem of isolating the tight junction integral membrane protein had become so notorious that a joke was going around: "The boss should not mention this theme for postdoctoral fellows." He and his wife, Sachiko, had established a procedure to isolate cadherin-based adherens junctions from rat livers (Tsukita and Tsukita, 1989) and were studying the proteins enriched in this fraction. Immunizing mice with this fraction would ultimately show that the adherens junctions were contaminated with a large supply of ZO-1 (Itoh et al., 1993), which, Tsukita reasoned, meant the preps were highly enriched for tight junctions.

But in three years of raising monoclonal antibodies with the prep, he says, "we did not obtain one that appeared to recognize integral membrane tight junction proteins. What did this mean?" One possible explanation was that tight junctions were formed by lipids, as some investigators had proposed. Tsukita's group, however, "believed in the 'protein theory,' based on Stevenson's pioneering work." Another explanation was that the rat junctions were not sufficiently immunogenic in mice.

New models; new proteins

Based on this latter idea, then graduate students Mikio Furuse and Toshiaki Hirase began isolating similar fractions from cows, pigs, hamsters, and hens. Their attempts established the "Furuse-Hirase rule" of the lab: the smaller the livers, the more pure were the junction fractions. Thus, newborn chick livers gave "very beautiful fractions." In the end, it would take 5,000 chick livers. "Luckily, Okazaki provides about 80% of Japan's chickens, so we got a very low price," says Tsukita.

Using this tight junction fraction, the team generated three monoclonal antibodies that recognized a 65-kD integral membrane protein—a protein that localized to endothelial and epithelial tight junctions (Furuse et al., 1993). The group cloned the protein, designated occludin, and depicted a model of the protein as having four transmembrane domains. Using the chicken cDNA of occludin to track down the mammalian occludins took another two years (Ando-Akatsuka et al., 1996).

When Tsukita's group, now based at Kyoto University School of Medicine

Downloaded from http://rupress.org/jcb/article-pdf/171/6/916/1321390/jcb1716916.pdf by guest on 23 June 2021

Text by Kendall Powell
kendallpowell@comcast.net

in Japan, created mouse epithelial cells lacking occludin, however, the cells still formed tight junctions (Saitou et al., 1998). This sent them back to the hunt for other integral membrane proteins from the tight junction fraction, using occludin as a probe. In 1998, Furuse, Tsukita, and colleagues identified the claudins, which also have four transmembrane domains but no sequence similarity to occludin (Furuse et al., 1998). Tsukita adds, "the identification of ZO-1, occludin, and claudins opened a new way to understand the barrier and fence properties of tight junctions in molecular terms." JCB

Anderson, J.M., et al. 1989. *J. Cell Biol.* 109: 1047–1056.
Ando-Akatsuka, Y., et al. 1996. *J. Cell Biol.* 133:43–47.
Farquhar, M.G., and G.E. Palade. 1963. *J. Cell Biol.* 17:375–412.
Furuse, M., et al. 1993. *J. Cell Biol.* 123: 1777–1788.
Furuse, M., et al. 1998. *J. Cell Biol.* 141: 1539–1550.
Itoh, M., et al. 1993. *J. Cell Biol.* 121:491–502.
Saitou, M., et al. 1998. *J. Cell Biol.* 141: 397–408.
Stevenson, B.R., and D.A. Goodenough. 1984. *J. Cell Biol.* 98:1209–1221.
Stevenson, B.R., et al. 1986. *J. Cell Biol.* 103:755–766.
Tsukita, S., and S. Tsukita. 1989. *J. Cell Biol.* 108:31–41.

Microtubules turn over rapidly

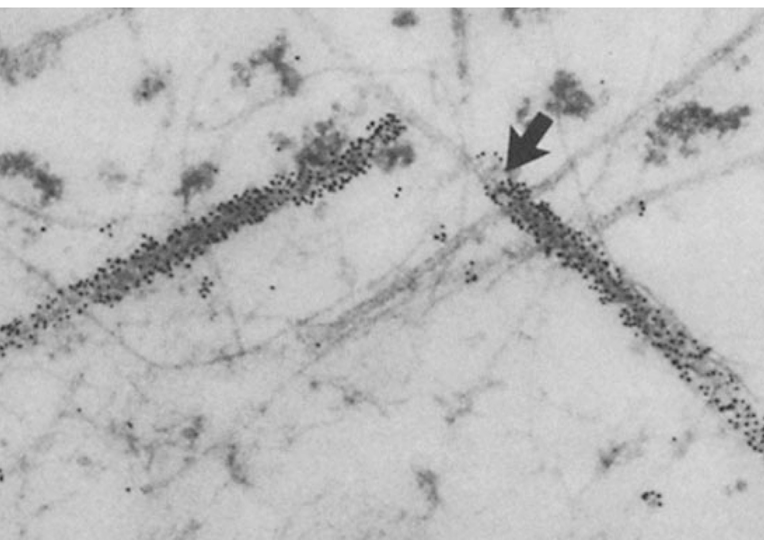
Eric Schulze and Marc Kirschner chemically label microtubules to define their dynamics.

Marc Kirschner recalls that the mid-1980s "was a very innovative time." While the era was giving rise to stonewashed jeans and rap music, cell biology was entering new territory as it finally went molecular. For cell biologists who, like Kirschner, were interested in the cytoskeleton, there was an even more exciting possibility: the modification of interesting proteins to form reagents that could be used to follow dynamics inside of cells. Kirschner's attention turned specifically to biotinylated tubulin.

By 1986, in vitro experiments had given rise to several models for microtubule (MT) dynamics, most notably treadmilling at steady-state (Margolis and Wilson, 1978) or the unusual growing and shrinking MT behavior termed "dynamic instability" (Mitchison and Kirschner, 1984). In a few preliminary in vivo experiments, it appeared that interphase MTs exchanged tubulin subunits rapidly, on the order of every 20 min (Salmon et al., 1984; Saxton et al., 1984), but without spatial resolution it was impossible to tell how they were being exchanged.

To address in vivo dynamics, Eric Schulze and Kirschner microinjected biotin-labeled tubulin into interphase fibroblasts for direct measurement of kinetics. With a growth rate of 3.6 µm/min, the team calculated that 80% of a cell's MTs would turn over in 15 min.

EM immunolabeling of the biotinylated tubulin allowed them to show that new subunit addition occurred in a directional manner, with one clear junction between unlabeled and labeled regions on all MTs (Schulze and Kirschner, 1986). The contiguity of old and new subunits demonstrated growth without the need for spontaneous assembly, and the speed and stochastic nature of the assembly (occurring at different times for different MTs) argued against universal treadmilling. Instead, the study's stunning images supported dynamic instability, with shrinking MTs providing the needed subunits for both growing MTs and new MTs being nucleated at the centrosome.


KIRSCHNER

Biotinylated tubulin (labeled) incorporates at microtubule ends.

"It was the first time you could actually look at single MTs and see that the tip of every MT was labeled," says David Drubin who was a postdoc with Kirschner around that time. "The static image of the cytoskeleton was falling away and that kind of rapid growth could only be explained by dynamic instability."

In a side note, the paper mentions the presence of a stable population of MTs, resistant to turnover. A subsequent study showed that the stable MTs are posttranslationally modified (Schulze et al., 1987), but Kirschner admits the role of this population remains a mystery. "Does the modification further stabilize the MTs?" he asks. "If MTs hit some region and become stabilized and modified, is this a way of generating polarity?" Some of the secrets of MT turnover, it seems, are yet to be revealed. JCB

Margolis, R.L., and L. Wilson. 1978. *Cell.* 13:1–8.
Mitchison, T., and M. Kirschner. 1984. *Nature.* 312:237–242.
Salmon, E.D., et al. 1984. *J. Cell Biol.* 99:2165–2174.
Saxton, W.M., et al. 1984. *J. Cell Biol.* 99:2175–2186.
Schulze, E., and M. Kirschner. 1986. *J. Cell Biol.* 102:1020–1031.
Schulze, E., et al. 1987. *J. Cell Biol.* 105:2167–2177.

Downloaded from http://rupress.org/jcb/article-pdf/171/6/916/1321390/jcb1716916.pdf by guest on 23 June 2021