Sticking it out with 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.

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 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).

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, Okayama 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...
Microtubules turn over rapidly

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

Text by Kendall Powell

FROM THE ARCHIVE • THE JOURNAL OF CELL BIOLOGY 917

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

Andersen, J.M., et al. 1989. J. Cell Biol. 109: 1047–1056.
Ando-Akatsuka, Y., et al. 1996. J. Cell Biol. 133:43–47.
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.

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.