Shoichiro Tsukita (1953–2005) – a cell biologist who will live with us forever

Sad news recently shocked the cell biology community deeply: on December 11, 2005, all too early, at only 52 years of age, Shoichiro Tsukita, a great scientist and good friend to many of us, died of one of the most devastating tumors, pancreatic carcinoma, more than a year after the initial diagnosis. I learned about his chemotherapy struggle and finally his death from Sachiko, his dear wife and colleague, and wondered for a while what we fellow cell biologists could say to her, his friends and colleagues in such a moment of grief. Well, there is one satisfying and pacifying truth: scientists of Shoichiro's eminence never really leave our world. Through their discoveries, they continue to be with us and with future generations of cell biologists for centuries to come. When a student first studies the important cellular structures apparent in life on this planet, (s)he will have to learn about the diverse kinds of tight junction, their molecular structure and their function, and will come across and remember Shoichiro's name.

Shoichiro Tsukita, Strasbourg, November 9, 2001, accepting the Honorary Membership of the German Society for Cell Biology, at the First Joint French-German Congress for Cell Biology, after his plenary lecture on 'Multifunctional Strands in Tight Junctions'.

Shoichiro Tsukita was born on July 7, 1953, in the harbor city of Kobe. He studied medicine at the University of Tokyo from 1971 to 1977 and, in 1981, obtained his PhD for a thesis on 'The morphological basis of fast axonal transport' based on work in Eichi Yamada's laboratory. He then became a lecturer at the same department and, in 1985, at the age of just 32, was made Director of the Department of Ultrastructural Research in the Tokyo Metropolitan Institute of Medical Science. In 1989, he moved to the National Institute for Physiological Sciences in Okazaki and at the same time became Professor at the Department of Medical Chemistry of Kyoto University. In 1996, he accepted an offer to be Full Professor and Head of the Department of Cell Biology of Kyoto University.

Shoichiro's view of the cell and his approach to understanding cells and their functions emerged from his deep-rooted interest in cell architecture and his wish to elucidate the molecular organization of cellular structures. His truly cell biological work, integrating biochemical, genetic and imaging approaches, was characterized by rigorous perseverance and stamina, coupled in his presentations with a classic Japanese artistic esthetic in his sense of light and electron microscopy. The vast majority of Shoichiro's more than 220 publications deal with cytoskeletal and membranous structures, culminating in a description of the molecular composition of tight junctions and the essential functions of their components. One can identify five periods in this epoch-making breakthrough research.

In 1989, 'the Tsukitas', Sachiko and Shoichiro, well described as a 'dream team' research couple, published a paper in which they reported the isolation and enrichment of adherens junctions from the bile canaliculi of liver tissue. This fraction soon turned out to be a gold mine, allowing the identification of a series of new adherens-junction-associated proteins, their function-related modifications and interactions with cytoskeletal proteins, including systematic molecular changes in carcinogenesis. In addition, they also noticed that this fraction provided a direct, albeit time- and energy-consuming, way to clarify the molecular composition of one of the most sought-after structures in the history of cell biology.

It was Shoichiro and his collaborators who in 1993 were the first to have the holy grail of membrane research in their hands, the lipid-buried proteins of the tight junction, the *zonula occludens*. With an admirably coherent strategy, but also – sorry, Shoichiro, you know that this is sheer admiration – brute force, power and labor, he and his colleagues identified, isolated, cDNA-sequenced and localized the first tight junction structural protein, which they called occludin. And by cDNA transfection of cultured fibroblasts, which lack such junctions, they elegantly proved that occludin can form the typical intramembrane ridges so characteristically seen in electron micrographs of rotary-shadowed freeze fractures through the lipid layers of the plasma membrane.

Shortly after, in 1998, Shoichiro noticed – surely disappointed at first – that occludin-deficient mice can still form tight junctional structures and retain many tight-junction-dependent functions. After the initial dispair, however, he – typically of Shoichiro – did not give up but went back to square one, the liver fraction. He soon found the deep molecular secret of tight junctions and their compositional diversity: a completely new protein family – the claudins – short, fourpass transmembrane proteins that number 24

to date. In the same year, he and his collaborators showed that claudins are sufficient to form typical tight junction structures, recruit occludin and specifically anchor all the proteins constituting the cytoplasmic plaque beneath.

As so often occurs in cell biology, the Tsukita laboratory had to learn that, what look like identical structures can be composed in different tissues of different members of a multigene family, apparently for evolutionarily advantageous reasons. The cell-type-specific claudin composition patterns not only distinguish the tight junctions of different tissues but also turned out to be key to understanding the claudins' many tissue-specific functions, as well as diseases resulting from loss of these.

I happen to know how surprised the Tsukita laboratory were – if not again initially disappointed – when they recognized that some of the claudin amino acid sequences they had just determined were already in the databases. This quickly turned into an 'eureka' moment, however, when they noticed that some of these sequences were known to be of receptors for bacterial enterotoxins such as *Clostridium perfringens* toxin, and this recognition led directly to novel explanations for the pathogenesis of various diseases, from toxically induced diarrhoeas to various kinds of edema. Subsequent studies of mice lacking specific claudin genes and experimental disruption of the tight sealing of the extracellular loops of claudins exposed the important roles of claudins in tissue formation and separation of liquid compartments in the body. In doing so, the work provided a general explanation for the flow of liquid and solutes in and out of tissues, as well as a potential molecular handle for interfering with – or sealing and healing – the membrane barrier that is essential for metazoan life. Since 2002, this concept has also included the tight junction systems of diverse stratified epithelia, going against the long-standing textbook dogma.

Tight junctions had one final surprise in store, however. Until this point, the Tsukita group had only discovered tight junction proteins at regions where two cells are coupled. In another experimental tour-de-force, they identified another new four-pass membrane protein, tricellulin, and showed that it localizes with flabbergasting topogenic specificity to contact sites where three cells meet. This elegant work revealed the final epithelial sealing element and was published in the last days of Shoichiro's life.

But one structure was never enough for this cell biology decathlete. Throughout the years, Shoichiro continued to discover new cytoskeletal and membrane-associated proteins of tight junctions and adherens junctions, notably members of the ezrin-radixin-moesin (ERM) family and associated molecules, and contributed to the elucidation of their functions. He never stopped after such basic research discoveries, but in lively, well-chosen collaborative projects he carried on the work to examine the developmental and pathological implications. Many of these findings became hallmark contributions to the etiology and diagnosis of various diseases.

Finally we should also gratefully acknowledge Shoichiro's great and global service to the cell biology community – as an organizer, tireless reviewer of grant proposals and manuscripts and editorial board member of many journals. *Journal of Cell Science* had the great fortune to benefit from this for over 15 years.

A life too short, but a life fulfilled for a full-time cell biologist. We honor Shoichiro and his work, and our hearts go out to his widow Sachiko, their son Kazuto and all our colleagues from the 'Tsukita lab'. We wish that they continue and extend what he began.

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