From the Archive

The sticky business of discovering cadherins

When scientists move from one lab to another, the inevitable change in reagents or mysterious new water properties can vex even the most robust of experimental set-ups. But for Masatoshi Takeichi, a change in the recipe for a trypsin solution when he moved from Kyoto University to the Carnegie Institution Department of Embryology eventually led to his seminal discovery of the cadherin family of adhesion molecules.

Takeichi started investigating the role for divalent cations in cell adhesion as a graduate student in Kyoto, where trypsin treatment of cells disrupted adhesion temporarily before cells reaggregated. When he moved to Carnegie for a fellowship, trypsin treatment led to permanent disruption of adhesion. It turned out that the Carnegie trypsin contained the calcium-trapping molecule, EDTA, as well as the trypsin enzyme. This combination gave Takeichi a clear picture of how to test for calcium-dependent adhesion.

He disrupted Chinese hamster V79 cells with EDTA alone, trypsin plus calcium, or trypsin plus EDTA and assayed for aggregation afterward. The results revealed calcium-independent and calcium-dependent adhesion pathways. More importantly, the calcium-dependent mechanism could be protected from trypsinization by calcium (Takeichi, 1977).

"It was so clear, then, that there was a very important calcium-dependent adhesion molecule," says Takeichi. "Therefore, I just thought I needed to identify that molecule." In a preliminary experiment in this study, he did identify a 150-KDa cell surface protein that disappeared from trypsin plus EDTA–treated cells. He remembers at least one reviewer's irritation at the premature correlation.

It would, however, be five more years (and a new cell system) before Takeichi could nail down the identity of the first cadherin molecule (Yoshida and Takeichi, 1982). He recalls several roadblocks while trying to develop an antibody from the V79 cells that could disrupt calcium-dependent adhesion and

be used to purify the protein. Then he happened to read a paper by Rolf Kemler using anti-serum made from whole F9 teratocarcinoma mouse cells (Kemler et al., 1977). 8-cell embryos treated with the anti-serum decompacted because the cells rounded up in a manner that was very similar to the behavior of Takeichi's trypsin plus EDTA–treated cells. Once he switched to the F9 cells, Takeichi identified E-cadherin as the first member of a protein family now known to regulate adhesion and, consequently, morphogenesis and tissue structure.

"Masatoshi is remarkable in his ability to use incomplete information in a complex problem to reach the right conclusion. Obviously, he has a very good feel for the biology involved," says Urs Rutishauser, who had identified another important adhesion molecule, NCAM, the year before (Rutishauser et al., 1976). (NCAM was soon found to be important in adhesion [Rutishauser et al., 1978a] and fasciculation [Rutishauser et al., 1978b] of chick neuronal cells.) He notes that although some peers might have worried about the "purely correlative information" that linked the 150-KDa protein to calcium-dependent adhesion, "nevertheless, he was correct."

Rutishauser, now at Memorial Sloan-Kettering Cancer Center (New York, NY), says a key component of the 1977 Takeichi study was the observation that calcium-dependent adhesion resulted in more than cells just sticking to each other—there were morphological changes in the adhering cells and their neighbors.

The cell spreading led Takeichi to predict, accurately, that calcium-dependent adhesion might regulate morphogenetic behavior of cells. In later studies he showed that cadherins adhered homophilically (Hirano et al., 1987), which he confirmed by transfection (Hatta et al., 1988), and were subject to phospho-regulation (Matsuyoshi et al., 1992).

The original 1977 paper "was so primitive," says Takeichi, now director of the RIKEN Center for Developmental Biology (Kobe, Japan). "But I'm proud of that paper because it was the beginning of the cadherin story." Prior to the discoveries of cadherin and NCAM as the first cell surface "receptors," the field had struggled to interpret conflicting data from temperature- and cation-dependent studies of adhesion. Now, as molecular cell biology techniques blossomed going into the early 1980s, they had specific molecules with which to track down adhesion pathways. **JCB**

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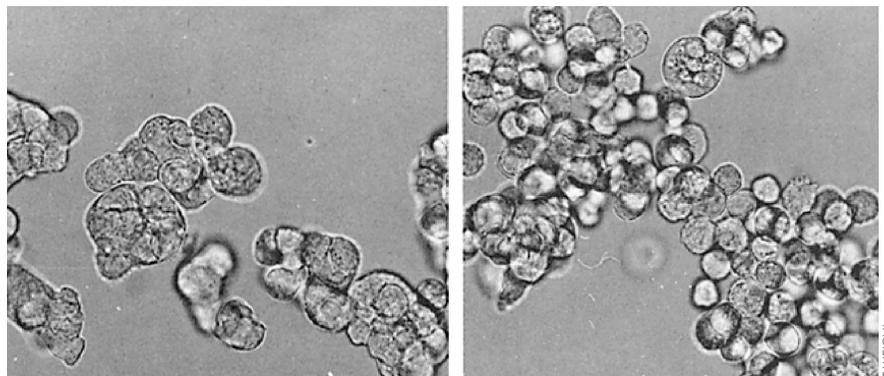Matsuyoshi, N., et al. 1992. *J. Cell Biol.* 118:703–714.

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Some cell–cell adhesion (left) disappeared in the absence of calcium (right).

TAKEICHI

Cell processes must be able to reach through filters to touch ECM for stimulation of collagen synthesis.

Contacting the matrix

In 1975, the extracellular matrix (ECM) and its potential as the "embryonic inducer" was just getting interesting, recalls Elizabeth Hay (Harvard Medical School, Boston, MA). Using an ingenious combination of biochemistry and tissue culture on the new Nucleopore filters, she and Stephen Meier would show that direct contact with the ECM was necessary for corneal epithelium to differentiate (Meier and Hay, 1975).

As early as 1955, Clifford Grobstein had proposed that the way one tissue induced another to develop might be through the presence of ECM (Grobstein, 1955). With his Millipore filter experiments he had shown that ECM alone could induce mouse salivary gland tissue to differentiate (Grobstein, 1953). A 1966 study showed that myoblasts plated onto collagen (then the known major component of ECM) would differentiate (Hauschka and Konigsberg, 1966). This was shocking to most researchers, Hay recalls, because, "at that time, ECM was believed to consist only of collagen and some proteoglycans. The idea that those things could be the embryonic inducer was repulsive to some because they thought it needed to be very specific."

The availability of Nucleopore filters with straight pores of varying diameters gave Meier, then a post-doc with Hay, a way to quantitate the level of contact between corneal epithelial cells grown on the top side of the filter and a killed lens ECM on the bottom. They also measured the level of differentiation using a biochemical assay of collagen synthesis. This set-up gave the team a definitive way to test whether physical contact with ECM or a diffusible molecule controlled induction.

By changing the pore size or using stacks of filters, the two were able to show that collagen synthesis by the epithelial cells increased as the pore size increased, and occurred only if the pores were big enough for the cells to make direct contact with the ECM deposited on the other side of the filter. Through this paper and further work, "it became obvious that there wasn't some specific and magical molecule coming from one tissue, but [development] could be influenced in different ways by the normal molecules cells were putting into the ECM," says Hay.

In 1981, the laboratory showed that soluble ECM components collagen, laminin, and fibronectin could direct the differentiation of corneal epithelium in vitro, whereas albumin, IgG, and glycosaminoglycans had no effect (Sugrue and Hay, 1981). In addition, the differentiation was matched to actin filament reorganization in the cell cortex. These demonstrations that matrix contact influenced cell behavior led logically to the search for and discovery of integrins, receptor molecules that connected ECM components to the cell's cytoskeleton (Tamkun et al., 1986). **JCB**

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Myosin powers cytokinesis

The stage was set in 1977 for Issei Mabuchi and Makoto Okuno to show that myosin interacted with actin to provide the force behind cell cleavage (Mabuchi and Okuno, 1977). Several groups had spotted nonmuscle myosin in a variety of cell types using electron microscopy and antibodies (e.g., see Fujiwara and Pollard, 1976). And numerous ultrastructural studies showed that the contractile ring contained microfilaments, which had been identified as actin in newt eggs, crane fly spermatocytes and human HeLa cells by the early 1970s. Furthermore, the force exerted at the cleavage furrow had been measured in echinoderm eggs as comparable to the tension in skeletal muscle (Rappaport, 1967).

Finally, Mabuchi himself had isolated myosin from the cortex of dividing sea urchin and starfish eggs (Mabuchi, 1973; Mabuchi, 1974). From all of this, the University of Tokyo duo concluded, "it would be reasonable to suspect that actin and myosin would interact to produce the force of constriction." To test this suspicion, Mabuchi says he decided the microinjection of myosin antibodies would be "the only promising method." He injected an antibody raised against starfish egg myosin into dividing eggs in the first experiment to use antibodies as protein inhibitors in live cells.

The antibody inhibited the actin-activated ATPase activity of myosin in a dose-dependent manner. And when it was injected into eggs during the second interphase, it stopped all subsequent cleavage events. Mitosis still occurred normally in most of the injected cells, even if cleavage was inhibited. The study solidified a role for actin–myosin contraction in cytokinesis but not nuclear division—and, equally important, a role for antibodies in disrupting cellular phenomena. **JCB**

Cytokinesis fails after injection of anti-myosin antibodies.

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