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 quantify 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).

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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