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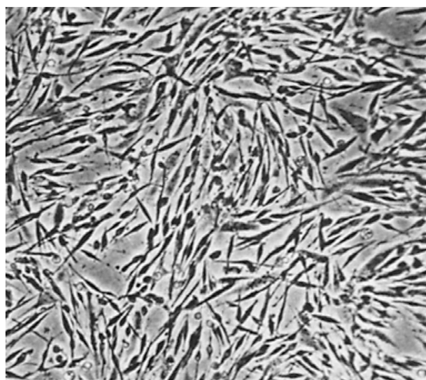
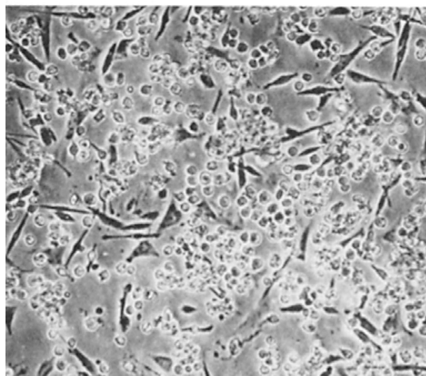
Pursuing the middleman: the hunt for integrins

By the mid-1970s, the idea of a transmembrane molecule linking the cytoskeleton to the extracellular matrix (ECM) had some supporting evidence. Adhesion molecules were hot in areas ranging from developmental biology to tumor biology, and the mounting suggestive evidence that a "fibronectin receptor" existed made it a "Holy Grail" for the next decade, recalls Richard Hynes, discoverer of fibronectin, in a memoir (Hynes, 2004).

Go monoclonal

Alan "Rick" Horwitz, then at the University of Pennsylvania in Philadelphia, had joined the gaggle of labs using antibodies to look for important cell adhesion players. But he was intimidated by the tedious, iterative method of the day in which labs generated polyclonal antibodies to cell surface molecules, screened them for the ability to disrupt adhesion, and then tried to narrow down antigens by successive purification and new antibody generation.





HORWITZ

An anti-integrin antibody (bottom) makes cells float.

Not only did the approach require too many resources for a relatively small lab, but Horwitz noted that some labs (including that of Clayton Buck at the Wistar Institute on the same campus) ran into dead-ends with the messy system, never reaching one clear antigen target molecule. When a colleague teaching with Horwitz told him of the new technique using hybridomas to generate monoclonal antibodies, "it was just obvious that it was the way to get these molecules, to get an adhesion-blocking antibody that was pure."

So his lab set to work immunizing mice with cell surface blebs of chick myoblasts. The resulting hybridoma supernatants were assayed for, among other things, their ability to detach myoblasts from the plastic culture dish. Horwitz recalls that a technician mistakenly thought one antibody was killing off the cells because they were rounding up and floating to the medium surface in such hoards. A quick investigation revealed that the detached myoblasts were alive and well and still able to fuse with each other in character with normal developing muscle cells. The antibody also released cells from an explant of chick embryo muscle, giving it in vivo significance. That antibody eventually became known as the cell substrate attachment antibody, or CSAT.

Antibody to antigen to clone

Knowing the Buck lab would be extremely interested and that it would speed the study along, Horwitz walked over to broker a collaboration. The study became one of the first to use a monoclonal antibody to disrupt a complex cellular process like adhesion and would eventually lead all the way to identification of the antigen molecule (Neff et al., 1982). But it wasn't until he saw the clean immunofluorescence of CSAT, reminiscent of adhesion plaque staining, that Horwitz realized CSAT's antigen was in fact a candidate for the transmembrane linker connecting actin to fibronectin (Neff et al., 1982).

This notion was strengthened by Caroline Damsky in the Buck lab, and by Wen-Tien Chen, at Georgetown University in Washington, DC, and Ken Yamada at the NIH. Chen and Yamada had been characterizing a similar cell-detaching monoclonal antibody, JG22, that they had obtained from another lab (Greve and Gottlieb, 1982). Both groups showed by immunofluorescence that their antigens lined up with fibronectin and actin (Chen et al., 1985; Damsky et al., 1985).

Richard Hynes got to see this "beautiful immunofluorescence," in this case generated by the CSAT antibody, during seminar visits in 1984. He was convinced that the CSAT antigen was the fibronectin receptor and was keen to collaborate since his group at the Massachusetts Institute of Technology (Cambridge, MA) was well-poised with an expression cloning system. They used the highly specific antibodies to clone the cDNA encoding the receptor, leading to the Buck-Horwitz-Hynes publication of the sequence of the β1 subunit of "integrin," the name finally coined by Hynes (Tamkun et al., 1986).

Binding proof

While the cloning was ongoing, direct binding between the CSAT antigen and fibronectin still had to be demonstrated. When Horwitz heard Yamada present his data on the low affinity of fibronectin for cells, he realized the ligand was probably falling off during column washing. He switched to a gel filtration strategy using buffer equilibrated with fibronectin so that the CSAT antigen always saw its ligand. Under these saturating conditions, the two molecules ran off the column bound together as a complex (Horwitz et al., 1985), a result confirmed independently by the Yamada lab (Akiyama et al., 1986). He and Buck used the same method to show that the antigen acted as a laminin receptor, and delivered a "home-run" by establishing the transmembrane link in vitro, with integrin binding both to the ECM fibronectin and to cytoplasmic talin (Horwitz et al., 1986).

During this time, Michael Pierschbacher working with Erkki Ruoslahti and the Yamada lab showed that peptides containing the sequence RGD comprised

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the recognition motif in fibronectin. Pierschbacher and Ruoslahti used this finding to isolate the fibronectin receptor using a modified affinity–column approach (Pytela et al., 1985).

Much work would come later, defining the roles of all of the ECM components, cytoskeletal players (Otey et al., 1990), and integrin subunits (e.g., Wayner and Carter, 1987), including those acting in lymphocyte adhesion (Jalakanen et al., 1987; Dustin and Springer, 1988; Diamond et al., 1990). But Buck notes that out of the 1982 CSAT paper "came definitive identification of the integrins as a complex linking the cytoskeleton to the ECM. It also led to the sequencing of the β1 subunit, and the field developed rapidly after that."

The search also integrated the Buck, Horwitz, Hynes, and Yamada laboratories into lasting friendships and collaborations. "We believed in what we did," Horwitz recalls. "We first had a handle on adhesion, and then neuronal outgrowth and cancer came into view. At one point, we thought we had the whole world." The field would go on to show that this receptor was part of a larger cell biology story—initiating cell signaling events central to many cell activities. JCB

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Frog egg extracts can do a cell's work

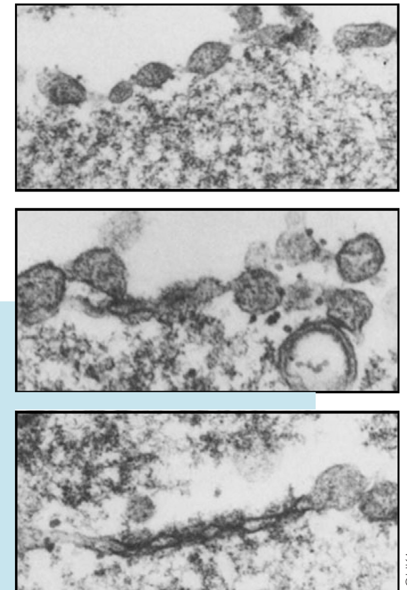
For his graduate project at the University of Toronto, Manfred Lohka wanted to answer one simple question: what enzyme was controlling the decondensation of a sperm nucleus when it entered the egg? At the time, an egg protease was thought to be involved, so he figured he should start by making egg extracts in which to test sperm. He got much more than he bargained for, including the birth of a potent cell-free biochemical assay.

After harvesting a test tube of eggs from *Rana pipiens* frogs, Lohka could spin the eggs at low speed and pop the cytoplasmic contents out of their plasma membranes "just like taking the skin off a grape." If he added *Xenopus laevis* sperm heads to the activated cytoplasm, he observed that the sperm heads transformed into pronuclei and then mitotic chromosomes (Lohka and Masui, 1983).

But it wasn't until Lohka and advisor Yoshio Masui looked at their extract-plus-sperm preparations by electron microscopy that they realized that not only did the sperm nucleus decondense, but a nuclear envelope was assembling around it as well (Lohka and Masui, 1984). Lohka provided one of the first descriptions of envelope assembly: membrane vesicles flattened and fused into a double-membraned structure, complete with nuclear pores. In addition, if Lohka fractionated the egg extracts with a higher spin and separated them into soluble and particulate fractions, he could show that envelope assembly required both.

Lohka credits Masui's love of unorthodox approaches for the discovery that egg cytoplasm can support cellular activities at least for short periods. "That was back when how you did experiments was an expression of your personality," says Lohka, now at the University of Calgary in Canada.

He notes that the study outcomes were not as important in the long run "as the notion that you could actually get quite complex cell processes to occur in cell-free extracts." The system was used to purify metaphase-promoting factor (Lohka et al., 1988), which led to the identification of cdc2 and cyclin as its components (Gautier et al., 1990) and the extracts became a valuable tool for further investigating the cell cycle (Murray, 1991). The system has also been used to study nuclear transport (Newmeyer et al., 1986), DNA replication (Mills et al., 1989), and spindle microtubule dynamics (Heald et al., 1996). JCB


LOHKA

Frog egg extracts allow the study of nuclear envelope formation.

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