When Caroline Damsky moved her lab to the University of California, San Francisco, in 1985, she knew she would have an instant colleague in Zena Werb. There was a natural connection between their work. That connection would be reflected in their discoveries, which delineated an integrin-mediated pathway from the extracellular matrix (ECM) outside the cell to the internal, gene-expressing life of the cell.

Werb and Damsky’s interests—metalloproteinase (MMP) gene expression and adhesion receptor antibodies—were both correlated with changes in cell shape and the actin cytoskeleton. Some dismissed the shape changes as simple, physical responses to a changing attachment environment, with no need to invoke signaling, but the two wondered if there was something more. “Were the cells getting signals from the ECM that could affect [protease] gene expression?” asks Damsky. “Zena was the doyenne of the protease side of things and I was the doyenne of the integrin side.”

Until this point, integrins had been viewed as being important primarily in “sticking cells down,” says Damsky. Her hunch was that integrin’s role went beyond glue to sending signals of some kind. At the same time, work paralleling Werb’s suggested that proteases had some part in regulating cell connections to the ECM. Damsky says it was only natural to combine forces.

When Damsky added her adhesion-blocking antibodies to fibroblasts in culture, thus specifically neutralizing a fibronectin receptor named \( \alpha_5 \beta_1 \) integrin, the cells started expressing collagenase and stromelysin, two ECM-degrading MMPs (Werb et al., 1986). Receptor clustering was needed: induction was increased when the antibodies were cross-linked and it did not occur with monovalent fragments.

Although native fibronectin did not induce protease expression, the authors showed that fragments of fibronectin, ranging from 120 kD to six amino acids, did. The fragments shared the RGD cell binding sequence characterized by Pytela et al. (1985). This suggested that degradation products of fibronectin, bound to integrins, could turn on protease expression.

“Cells were recognizing different states of the matrix molecule,” says Werb. “Embedded in a molecule was both the mechanism for homeostasis as well as for cell migration and changes that might occur, for example, in wound healing.” The idea fit with emerging studies showing distinct responses to different fibronectin fragments. For example, human monocytes had chemotactic activity to the cell-binding domain of fibronectin, but not to the intact molecule (Clark et al., 1988).

Damsky notes wryly that although they were first to demonstrate integrin signaling, integrins became “respectable” signaling molecules after the labs of Joan Brugge, Thomas Parsons, and Lewis Romer showed that integrin ligation triggered the phosphorylation of focal adhesion kinase (Lipfert et al., 1992; Burridge et al., 1992). Nevertheless, the Werb/Damsky collaboration clearly set up a very large field, says Werb. She notes with pride that their work appears at the top of the list of 3,488 papers pulled up by a search for “integrin signaling.”


Microtubules get dynamic

Microtubules were seen first as static struts in EM images (see “Microtubules get a name” JCB 168:852) and then as polymers created from a defined protein component (see “The discovery of tubulin” JCB 169:552). It took a series of studies in the 1980s to emphasize that dynamics were essential for microtubule action in the cell.

First, in vitro polymerization was needed as proof that there was no additional magic ingredient needed for microtubule formation. That achievement was forthcoming once EGTA was added to the mix to get rid of inhibitory calcium (Weisenberg, 1972). But this didn’t exactly bring hordes rushing into the field of in vitro microtubule polymerization. Summers and Kirschner (1979) did find that microtubule growth was polar, occurring more readily at one end of the polymer than another. But it would be another 12 years after the initial in vitro method was reported before detailed in vitro studies led to the landmark theory of dynamic instability (Mitchison and Kirschner, 1984). The phenomenon was later observed in vivo in real time (Cassimeris et al., 1988).

The discovery of dynamic instability, in which microtubules grow persistently but suffer stochastic switches to catastrophic shrinkage, was only possible because of the observations of the dynamics of individual microtubules. Initially, says Tim Mitchison of Harvard Medical School, “the microtubule mafia were totally surprised and didn’t really believe it.” If it was true, however, then these individual behaviors must be reshaping bulk microtubule populations in vivo. Just a month after Mitchison and Kirschner’s paper, there were two reports indicating how important turnover and dynamic instability might be for the cell. Salmon et al. (1984) found that the half-time for spindle microtubule turnover (based on fluorescence recovery) is only ~19 s, and Saxton et al. (1984) reported that microtubules turn over in minutes during interphase but seconds during mitosis.

One way that the turnover could be put to work is via microtubule flux—the poleward movement of microtubule subunits resulting from depolymerization at the pole balanced by polymerization at kinetochores. Bleaching experiments showed no sign of flux (Salmon et al., 1984), but it was possible that the rapid turnover of nonkinetochore microtubules was obscuring the flux of the less dynamic kinetochore microtubules. A direct demonstration of flux came when Mitchison (1989) reversed the contrast by making a photoactivatable, fluorescent derivative of tubulin. Encouraged by chemist David Trenton, “it was a fairly simple extension to think of turning fluorescence on instead of off,” he says.

The attached chemical group became fluorescent only when illuminated by light of a particular frequency. Mitchison used this light to mark a bar of fluorescence on an otherwise nonfluorescent spindle. Initially the bar faded as nonkinetochore microtubules turned over, but the remaining fluorescence moved steadily poleward. A flux-based force is still thought by many to contribute significantly to the poleward movement of chromosomes during anaphase (Rogers et al., 2004).