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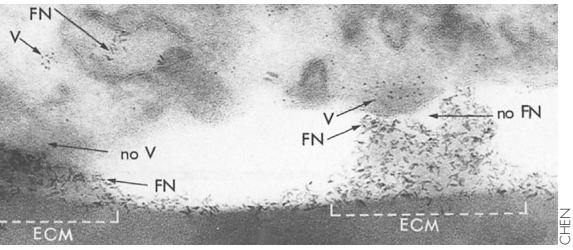
More than one way to attach

How cells connect to each other and to the extracellular matrix (ECM) was a sticky issue in the early 1980s. Integrins, molecules that hook the cytoskeleton to ECM proteins such as collagen and fibronectin, hadn't been discovered, but evidence for a link between external and internal fibers was mounting. For example, Irwin Singer (1979) observed that extracellular fibronectin molecules closely approached—or possibly attached to—intracellular actin. Several researchers postulated that membrane-spanning receptors made the connection. A pair of papers by post-doc Wen-Tien Chen of the University of California, San Diego, and his adviser S. Jonathan Singer bolstered the idea that cells deploy different membrane receptors to couple with different components of the matrix.



CHEN

Different cell adhesion sites have different arrangements of vinculin (V) and fibronectin (F).

A new technique devised in Singer's lab gave the researchers a clearer look at the junction between cell and surface. They reared cells on a gelatin mat, which they could roll up like a carpet, freeze, and cut into thin slices. Staining the gap with two types of antibodies pinpointed proteins clustering on both sides of the membrane. When the researchers zoomed in on a type of contact called a focal adhesion, they saw no signs of fibronectin outside the cell, although it's a key component of some cell surface junctions (Chen and Singer, 1980). Fibronectin's absence meant that cells needed a second kind of receptor to attach to the extracellular fibers found in focal adhesions, the researchers hypothesized.

A follow-up study that included more kinds of contacts (Chen and Singer, 1982). They found that fibronectin amassed in two kinds of interactions, but not in two others. Moreover, at one type of fibronectin-rich junction, microfilaments inside the cell ran parallel to the membrane. But in another sort of interaction devoid of fibronectin, microfilaments attached to the membrane head-on, like an extension cord plugging into a wall socket. These structural differences solidified the case that cells carry different receptors for different extracellular matrix proteins, says Chen (now at the State University of New York, Stony Brook). One type fastens fibronectin to microfilaments stretching along the membrane; the other joins other extracellular proteins to microfilaments that arrive perpendicular to the membrane. Chen then teamed with Kenneth Yamada of the National Cancer Institute to characterize a fibronectin-grabbing receptor (Chen et al., 1985), which later work identified as an integrin. **ML**

Chen, W.-T., et al. 1985. *J. Cell Biol.* 100:1103–1114.
Chen, W.-T., and S.J. Singer. 1980. *Proc. Natl. Acad. Sci. USA.* 77:7318–7322.
Chen, W.-T., and S.J. Singer. 1982. *J. Cell Biol.* 95:205–222.
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Dishing up bone formation

Hiroaki Kodama knew little about dental and bone research when he became an assistant professor at Tohoku Dental University. What he *did* know was cell culture technology, and he recognized the field's need for a clonal cell line "which retains as far as possible a normal ability to differentiate into functional cells."

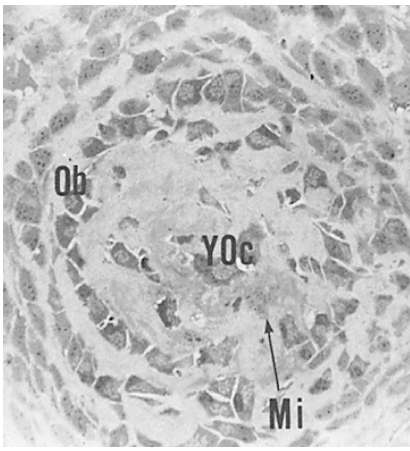
In 1979, he and his colleagues started establishing cell lines that differentiated into osteoblasts (bone-forming cells) rather than odontoblasts (dentin-forming cells). At that time, only a few primary bone cell cultures had been reported to show hints of in vitro bone formation (Binderman et al., 1979; Nijiweide et al., 1982).

Kodama (RIKEN, Wako Saitama, Japan), says the secret to success was using the same cell culture method used to make the immortalized mouse fibroblast 3T3 cell line (Todaro and Green, 1963; see "A cell line that is under control" *JCB* 168: 988). This meant repeated subcultivation of newborn mouse skull bone cells under 3T3 conditions—3 days to transfer at a factor of 3 cell plating density.

When one of the lines that arose, called MC3T3-E1, became confluent, it exhibited properties of osteoblasts, including high alkaline phosphatase activity and staining for calcified secretions (Sudo et al., 1983). At day 21 of culture, calcified nodes appeared and then grew in number and size to eventually fuse with one another. By day 30, white nodes in the dish were visible to the naked eye. But because calcium is easily deposited under basic culture conditions, the team had to show that they were seeing true bone formation.

Mineralization proceeded in much the same way it did in vivo, by the secretion of matrix vesicles containing crystals, which were deposited along collagen fibrils. Electron diffraction defined the crystals as hydroxyapatite, the chemical that forms bone matrix.

But in vitro bone formation, Kodama notes, was not easily reproducible by other groups until the discovery that bone morphogenetic protein acts as a potent inducer of osteoblast differentiation (Yamaguchi et al., 1991). The MC3T3 cell line remains an important tool for studying bone cell differentiation today, with renewed focus on mesenchymal stem cell differentiation and the need for better osteoporosis therapies, like bone-building agents. In fact, the line was the most widely distributed by the RIKEN Bioresource Center (Tsukuba, Japan) in 2003. **KP**



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Making bone in a dish: osteoblasts (Ob) surround a nodule of young osteoclasts (YOc) embedded in mineralized matrix.

Binderman, I., et al. 1979. *Science.* 206:222–225.
Nijiweide, P.J., et al. 1982. *J. Cell Biol.* 93:318–323.
Sudo, H., et al. 1983. *J. Cell Biol.* 96:191–198.
Todaro, G.J., and H. Green. 1963. *J. Cell Biol.* 17:299–313.
Yamaguchi, A., et al. 1991. *J. Cell Biol.* 113:681–687.