

From the Archive

Dying on cue

William Earnshaw and colleagues wanted to plumb the intricacies of cell birth, but they ended up discovering a vital tool for studying cell death in vitro (Lazebnik et al., 1993). The sequence of events in apoptosis remained uncertain at the time, recalls Earnshaw (now at the University of Edinburgh in the UK), because suicidal cells die asynchronously. “You could never have a tube of cells all undergoing apoptosis at the same time,” he says. This made it difficult to pinpoint the biochemical details of each step.

Earnshaw’s group was hoping to crack a different

question: how the cell’s chromatin condenses during mitosis. To study the process, they had devised a cell-free system containing cytoplasm from dividing liver cancer cells. Nuclei bathed in these extracts appeared to begin mitosis—their DNA clumped against the nuclear membrane, for example.

But Earnshaw’s post-doc Yuri Lazebnik (now at Cold Spring Harbor Laboratory in New York) happened to attend a seminar on apoptosis and recognized similarities between dying cells and his isolated nuclei. Back in the lab, experiments confirmed that nuclei incubated in the cell extracts were following the script for apoptosis, not girding for division. Just as in apoptosis,

Integrin signal transduction

A cell resting on the extracellular matrix (ECM) doesn’t just sit there like a football fan in a La-Z-Boy. It develops a deep connection with its substrate. Contact between matrix proteins and integrin receptors in the membrane adjusts the cell’s cytoskeleton and shape (Haimovich, et al., 1993), galvanizes survival-promoting pathways, and causes numerous other changes. A 1992 paper by Keith Burridge, Christopher Turner, and Lewis Romer (Burridge et al., 1992) implicated the focal adhesion kinase (FAK) as a key relay for ECM signals. As later studies showed, FAK is a well-connected protein that gets involved in everything from the cell cycle to apoptosis.

By 1992, evidence indicated that ECM proteins pass their messages to the cell by tweaking integrins (see “ECM signals ECM degradation” *JCB* 172:642), but cell biologists had worked out only a few of the following steps. Integrins gather at focal adhesions, specialized portions of the membrane where the cell meets the matrix. Researchers had identified several

possible relay molecules at these junctions, including FAK (Schaller et al., 1992).

To probe FAK’s activity, Burridge and colleagues grew cells on different substrates and tested for proteins phosphorylated on tyrosine, an indicator of activation. The team found that two phosphorylated proteins abounded in cells reared on fibronectin—an ECM component and integrin ligand—but not in cells raised on plastic. One of these proteins, the researchers demonstrated, was FAK (Burridge et al., 1992). The other was paxillin, which later research linked to cell movement. Lipfert et al. (1992) observed a similar pattern of phosphorylation in platelets that snuggle up to the clot protein thrombin.

When Burridge and colleagues dosed cells with herbimycin A, which blocks phosphorylation of tyrosines, they noted fewer focal adhesions and fewer of the polymerized actin filaments that normally attach to these adhesion sites. Those results suggest that FAK responds to integrin stimulation by helping to mold focal adhesions and modify the actin cytoskeleton,

says Romer (now at the Johns Hopkins School of Medicine in Baltimore, Maryland). Burridge went on to show that the molecular switch called Rho spurs formation of focal adhesions by increasing the contractility of actin fibers (Chrzanowska-Wodnicka

and Burridge, 1996).

Meanwhile, other experiments have revealed that FAK’s influence extends to cell spreading and movement (Romer et al., 1994; Gilmore and Romer, 1996; Yano et al., 2004), proliferation (Zhao et al., 1998), and survival (Frisch et al., 1996). All of these functions involve integrins. Furthermore, research by Turner (now at the SUNY Medical Center in Syracuse, New York) and colleagues indicated that paxillin, the other focal adhesion protein phosphorylated in the original work, forms a signaling complex that helps instigate cell spreading and motility (West et al., 2001; Brown and Turner, 2004). **ML**

Brown, M.C., and C.E. Turner. 2004. *Physiol. Rev.* 84:1315–1339.

Burridge, K., C.E. Turner, and L.H. Romer. 1992. *J. Cell Biol.* 119:893–903.

Chrzanowska-Wodnicka, M., and K. Burridge. 1996. *J. Cell Biol.* 133:1403–1415.

Frisch, S.M., et al. 1996. *J. Cell Biol.* 134:793–799.

Gilmore, A.P., and L.H. Romer. 1996. *Mol. Biol. Cell.* 7:1209–1224.

Haimovich, B., et al. 1993. *J. Biol. Chem.* 268:15868–15877.

Lipfert, L., et al. 1992. *J. Cell Biol.* 119:905–912.

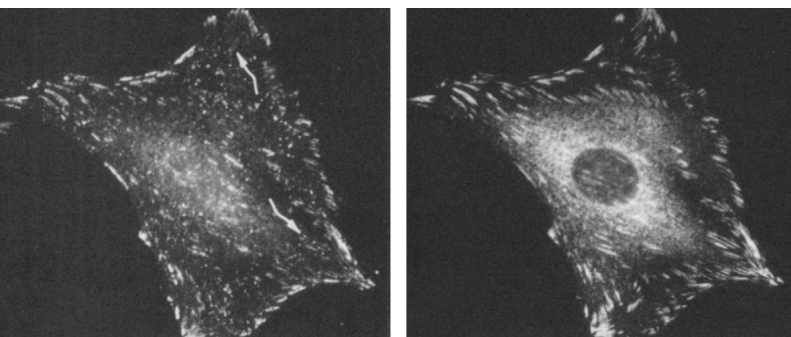
Romer, L.H., et al. 1994. *Mol. Biol. Cell.* 5:349–361.

Schaller, M.D., et al. 1992. *Proc. Natl. Acad. Sci. USA.* 89:5192–5196.

West, K.A., et al. 2001. *J. Cell Biol.* 154:161–176.

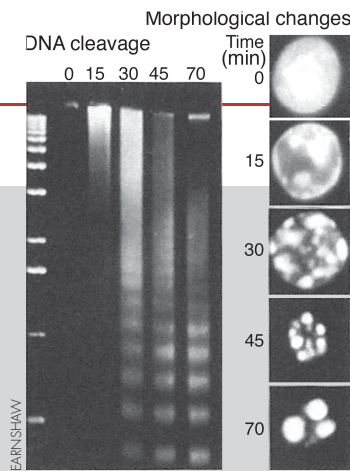
Yano, H., et al. 2004. *J. Cell Biol.* 166:283–295.

Zhao, J.-H., et al. 1998. *J. Cell Biol.* 143:1997–2008.



Proteins with phosphorylated tyrosines (left) match the location of focal adhesions (right).

BURRIDGE



Placing a nucleus in a cell extract causes the DNA to fragment, as during apoptosis.

DNA within the nuclei got minced into pieces that were multiples of 200 base pairs in length—the result of enzymes cutting between the nucleosomes. As in a dying cell, the nuclear membrane blebbed and extruded dense balls of chromatin. And the researchers found that zinc, which can stall

apoptosis, prevented the nuclei from deteriorating.

The results were important because they allowed researchers to create synchronized systems to study how protein-slicing enzymes such as the caspases orchestrate apoptosis, says Earnshaw. His group was the first to capitalize on this new ability (Lazebnik et al., 1994), identifying the specific amino acid sequence where caspases clip the DNA repair protein PARP. But if Lazebnik hadn't gone to that seminar, says Earnshaw, the researchers might still think that they had been looking at mitosis. **ML** Lazebnik, Y.A., et al. 1993. *J. Cell Biol.* 123:7–22. Lazebnik, Y.A., et al. 1994. *Nature.* 371:346–347.

Hold on for dear life

Some cells can't bear to leave home. As Steve Frisch (now at West Virginia University in Morgantown) and Hunter Francis reported in 1994, epithelial cells that lose touch with the extracellular matrix (ECM) kill themselves, a phenomenon the scientists dubbed "anoikis." Although other researchers were skeptical at first, this type of apoptosis turned out to be an important mechanism for managing cell numbers, preventing abnormal growth, and squelching cancer.

Frisch and Francis weren't searching for new cell death pathways when they chanced on anoikis. The pair was scrutinizing a bizarre adenovirus protein called E1a that can restore tumor cells to normal behavior. The researchers observed that tumor cells "reverse transformed" by E1a die when they separate from the ECM. E1a also bestows some epithelial characteristics on the reverse transformed cells, so Frisch and Francis decided to test whether disengagement from the ECM is fatal for normal epithelial cells.

They transferred normal epithelial cells to culture dishes coated with a compound that prevents cellular attachment. On gels, DNA from the free-floating cells showed a "ladder" pattern of equal-sized snippets, a telltale sign of the DNA degradation that occurs during apoptosis (Frisch and Francis, 1994). The researchers also detected breakdown products of the nuclear lamina, a sign that the nuclear envelope was disintegrating, another hallmark of apoptosis.

To confirm that separation from the ECM triggered death, the researchers raised cells on a congenial surface and then added small proteins that obstructed integrins, the membrane receptors that interact with the ECM. The cells died in droves. Frisch and Francis also showed that transforming cells with an oncogene or turning up production of the antiapoptotic protein bcl-2 staved off anoikis. The results "confirmed that epithelial cells are programmed to undergo apoptosis if they lose contact with the matrix," says Frisch. The researchers chose a Greek term mean-

ing "homelessness" for the phenomenon. Meredith et al. (1993) discovered a similar mechanism in isolated endothelial cells.

Eliminating wayward epithelial cells makes sense, Frisch explains, because they might grow in an inappropriate location. Still, they had to win over skeptical reviewers—it took more than a year to convince a journal to publish the report, he remembers.

Researchers have since learned that anoikis is ubiquitous. It helps cull excess cells in the digestive system (Hall et al., 1994), keeps the milk-producing bulbs in mammary tissue open (Debnath, et al., 2002), and helps hollow out the embryo early in development (Coucovanis and Martin, 1995). Other scientists

are piecing together how detachment causes death. For example, when cells break away from the ECM, they unshackle a protein called Bmf that detains survival-promoting molecules in the cell (Puthalakath et al., 2001). Frisch and colleagues showed that they could quell anoikis by inducing cells to produce a hyperactive version of the protein FAK, which flips on when cells attach to the ECM and promotes growth and survival (Frisch et al., 1996). This discovery was telling,

Frisch says, because tumors often pump out extra FAK (Agochiya et al., 1999), suggesting that many cancer cells can't leave home without it. Other tumors ramp up production of TrkB, a protein that helps nurture nervous system cells, and recent work suggests TrkB allows intestinal cells to elude anoikis (Douma et al., 2004). **ML**

Agochiya, M., et al. 1999. *Oncogene.* 18:5646–5653.

Coucovanis, E., and G.R. Martin. 1995. *Cell.* 83:279–287.

Debnath, J., et al. 2002. *Cell.* 111:29–40.

Douma, S., et al. 2004. *Nature.* 430:1034–1039.

Frisch, S.M., and H. Francis. 1994. *J. Cell Biol.* 124:619–626.

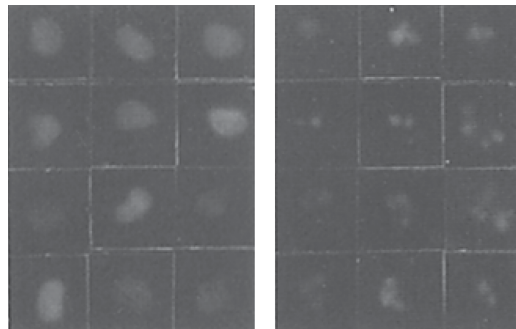
Frisch, S.M., et al. 1996. *J. Cell Biol.* 134:793–799.

Hall, P.A., et al. 1994. *J. Cell Sci.* 107:3569–3577.

Meredith, J.E., Jr., B. Fazeli, and M.A. Schwartz. 1993.

Mol. Biol. Cell. 4:953–961.

Puthalakath, H., et al. 2001. *Science.* 293:1829–1832.



The nuclei of epithelial cells (left) fragment when matrix contact is lost (right).