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).
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 confers 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 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.

Researchers have since learned that anoikis is ubiquitous. It helps curb 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 (Coucouvanis 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).

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An unattached kinetochore screams “Wait!”

In August of 1993, Conly Rieder and Greenfield “Kip” Sluder skipped a few sessions of the annual Microscopy Society of America meeting in Cincinnati, Ohio, to visit the Air Force museum in Dayton and see the bombers on display. During the drive, they discussed a topic that had been in the back of both of their minds.

Rieder’s story began with a 1988 call from Leland Hartwell, shortly before Hartwell had put forward the idea of cell cycle checkpoints (Hartwell and Weinert, 1989). Hartwell asked whether anyone had definitively shown that cells delayed anaphase until all chromosomes were hooked up to the spindle. Rieder noted that there was one obscure abstract concluding that newt cells never started anaphase in the presence of a monooriented chromosome (Zirkle, 1970). And Sluder, as a graduate student, had found that inhibiting spindle assembly delayed anaphase onset in sea urchin eggs (Sluder, 1979). But although there were some anecdotal reports of a chromosome attachment or spindle assembly checkpoint in other cell types, a proper study had not been done.

Now, the two road-trippers thought, was the perfect time to test the idea in mammalian somatic cells before someone else did. Rieder already had an undergraduate, Adriene Schultz, collecting video data on 126 individual PtK1 cells, which remain flat through mitosis and have 12 easy-to-follow chromosomes. She was measuring the time from nuclear envelope breakdown to anaphase onset and how long the cell had unattached kinetochores. The data lined up on a near-perfect linear regression (Rieder et al., 1994).

In addition, the group determined that a single unattached kinetochore was enough to delay anaphase. But once the last kinetochore attached to the spindle, anaphase would always proceed ~20 min later. Sluder suggested using the drug Taxol to artificially delay microtubule attachment for up to three hours. These cells also entered anaphase when the last kinetochore joined up.

“One unattached kinetochore was doing something that shuts down the whole spindle system,” says Sluder. According to Rieder: “The next question was, what does the cell monitor? Either it was monitoring bipolar attachment [of each chromosome] or the unattached kinetochore is screaming ‘Wait!’ through negative feedback.”

To test what the checkpoint monitored, the group relied on a handy laser set-up. Rieder had installed it to do chromosome microsurgery for aster ejection force studies. But he now found that ablating the centromere of the last unattached chromosome—to destroy either the entire centromeric region or just the unattached kinetochore—could override the checkpoint (Rieder et al., 1995). Blasting off a kinetochore on one of the already attached chromosomes to create a new monooriented chromosome did not delay the cell cycle, however. This indicated that the inhibitory signal was coming from the unattached kinetochore itself.

Sluder and Rieder went on to show that in cells with multipolar spindles any unattached chromosome pair would delay the whole system (Sluder et al., 1997), although in fused cells with two spindles the inhibitory signal was specific to the spindle with the unattached kinetochore (Rieder et al., 1997). By then, multiple spindle assembly checkpoint proteins, such as Mad2, had been identified, and they began to show up at the kinetochore (Chen et al., 1996). A literature battle waged for a while about whether the checkpoint was relieved by tension on the bioriented kinetochore or microtubule occupancy of the kinetochore. Occupancy won out when Mad2 binding was shown to be dependent on accumulated microtubules, although tension probably plays an important upstream role (Nicklas et al., 2001).

Chen, R.H., et al. 1996. Science. 274:242–246.
Hartwell, L.H., and T.A. Weinert. 1989. Science. 246:629.634.
Nicklas, R.B., et al. 2001. J. Cell Sci. 114:4173–4183.
Rieder, C.L., et al. 1994. J. Cell Biol. 127:1301–1310.
Rieder, C.L., et al. 1995. J. Cell Biol. 130:941–948.
Rieder, C.L., et al. 1997. Proc. Natl. Acad. Sci. USA. 94:5107–5112.
Sluder, G. 1979. J. Cell Biol. 80:674–691.
Sluder, G., et al. 1997. J. Cell Sci. 110:421–429.
Zirkle, R.E. 1970. J. Cell Biol. 47:235a

The first forty years

After highlighting papers from the first 40 years of the JCB, this issue marks the completion, at least for now, of the “From the Archive” series. We hope you will use this series as a sampler, and continue to enjoy the astonishing work contained within the entire online JCB archive.