Jeff's view

The art of stepping back

Scientific breakthroughs have their own punctuation. Some come with a triumphant exclamation mark because they explain something that had always puzzled us. The discoveries of the DNA double helix and of reverse transcriptase had *eureka*! written all over them. Other breakthroughs have an expectant question mark because they give us a novel tool, such as protein sequencing or the polymerase chain reaction, that makes our heart beat faster. And then there are those breakthroughs that come in quiet brackets. It would embarrass me to count those I overlooked. When I finally recognize their import, I always feel a little chastened. The Mitchell hypothesis and ATP-dependent proteolysis both made unobtrusive entrances. The same is true for the discovery that cells can kill themselves.

That life and death go together, even require one another, is not a new insight. Persephone, who reigned over the dead, was the daughter of Demeter, goddess of life-bringing harvests. But most Western societies see life as active and death as passive. Death is *suffered* or *inflicted*, be it by age, disease, starvation, or violence. It is outside chaos vanquishing the order of life. Biochemistry and cell biology have reinforced this lopsided view. They showed us that living cells are immensely complex and that cell death by starvation, heat, mechanical injury, or poison wreaks collapse of transmembrane ion gradients, swelling of the cell and its mitochondria, general loss of cell constituents, and inflammation of surrounding tissue. Something to be fought all cost. As Dylan Thomas put it: "*Do not go gentle into that good night!*".

Life has always faced chaotic death and learned to cope with it. Many organisms produce a grotesque surplus of germ cells or offspring so that a few of them may survive. Evolution has taught these parents that most of their progeny would die a chaotic death, so they overwhelm this death by plenitude.

But now we know that there also is another death, that cells can commit suicide in an orderly way. This 'programmed cell death' or 'apoptosis' is neither passive nor chaotic; in fact, it is anything but. Apoptosis shows us death's second face, and it looks like that of life.

Watching a cell commit suicide is like watching a well-rehearsed ritual. The cell shrinks; its mitochondria disintegrate; the plasma membrane forms blebs and breaks up into vesicles, which retain all of the cell's contents; and finally the vesicles are eaten by phagocytes. There is neither inflammation nor necrosis. The dying cell neatly seals itself into garbage bags, does not pollute the neighborhood, and avoids public disturbance. It disappears without a noise.

The molecular workings of this harakiri process are no less impressive. Apoptosis-regulating proteins form pores in the mitochondrial outer membrane, allowing proteins to escape from the intermembrane space into the cytosol. Several of these escapees are hydrolase activators or active hydrolases, which trigger dismantling of the cell. For example, cytochrome *c* and a cytosolic protein nicknamed *Apaf-1* assemble with the inactive pro-form of the cytosolic protease caspase 9 into a huge, wheel-shaped 'apoptosome', in which the proprotease is activated. Caspase 9 then triggers the proteolytic maturation of pro-caspase-3 and other caspases, which degrade the cell's cytoskeleton and induce blebbing of the plasma membrane and breakup of the cell into smaller vesicles. The apoptosome also appears to activate nucleases that split up the cell's DNA into characteristic nucleosomal fragments.

Somewhere along the way, phosphatidyl serine appears on the outer monolayer of the plasma membrane, perhaps because the ATP-driven 'flippase' that normally moves this phospholipid to the inner monolayer shuts down. Phosphatidyl serine serves as bait for roving phagocytes, which home in on the vesicles, devour them, and release anti-inflammatory signals that keep everything quiet. This basic machinery has been remarkably conserved from simple worms (in which some of its parts were first discovered) to man. All multicellular eukaryotes seem to have it. Unicellular eukaryotes generally lack the full-fledged machinery, except perhaps for some that parasitize more complex organisms. Nobody has so far found apoptosis in a bacterium.

There are many variations, footnotes and uncertainties to this basic picture. What sets off the process? There are many ways to do it, and the more complex an organism, the richer the triggering repertoire of its constituent cells. The initial signal can come from the outside, or from within the cell itself. Many external 'death signals' are proteins that bind to 'death receptors' on the target cell's surface. This interaction induces a regulatory protein to move from the cytosol, the cytoskeleton, or another intracellular parking spot to the integral outer membrane protein *Bcl-2*, thereby creating a pore in the outer membrane. The regulatory molecule usually is similar to *Bcl-2* that normally sits in the outer membrane, but it generally lacks a hydrophobic membrane anchor and a few other parts. The worm *Caenorhabditis elegans* has only a single *Bcl-2*-like regulatory protein; mammals have more than a dozen with which they can booby-trap their cells in many different places. Cells can also trigger apoptosis themselves: if the mitochondrial inner membrane starts to leak ions because of oxidative stress, if the cell's DNA has been damaged, or if some other vital indicator is going south. The biochemistry of the process gets more complex by the week and so does the literature on it, which still increases exponentially. But all of this bewildering information is held together by beautiful genetic experiments in worms, fruit flies and mice, which define the order of steps, their relevance, and sometimes their redundancy. Male *C. elegans* worms form 1179 somatic cells, of which 148 are condemned to die by apoptosis. For the hermaphrodites, the numbers are 1090 and 131. This simple worm has 13 apoptosis genes, all but two of them acting within every somatic cell.

How many genes control apoptosis in humans? The latest official tally has broken the one hundred barrier and I would not be surprised if the final number turned out to be several times higher. These genes control phosphorylations and dephosphorylations, limited proteolysis, intracellular protein

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traffic, wheels within wheels, you name it. The clockworks for orderly death and orderly growth are very much alike. The resemblance is more than coincidental, because apoptosis plays a pivotal role in the development and growth of complex organisms. When I was still in my mother's womb, it sculpted the fingers on my emerging hands; later on, it helped set up the wiring of my brain; still later, it delivered me from immune cells that would have turned against me; and all along the way, it cleansed my body of cells whose growth clock had gone awry. I hope that programmed death will continue to look after me. May it be alive and kicking until the end of my days!

Unlike chaotic death, programmed death can be retarded or even blocked by specific mutations. Some of these mutations inactivate an essential death gene. If they lead to cancer, the affected gene is formally a tumor suppressor gene. Other mutations upregulate a death-inhibiting gene. They, too, can result in cancer, making the affected gene a proto-oncogene. When we try to kill cancer cells, we usually resort to poison or radiation and inflict grave collateral damage. But if we knew the secret password for triggering apoptosis in cancer cells, we could simply ask these cells to please leave. I expect to see a host of new drugs that save human life by triggering cellular suicide. It boggles my mind.

Programmed cell death raises many profound questions; philosophers should fall all over themselves staking out this intellectual gold mine. I am intrigued by the fact that full-fledged apoptosis only appeared with the advent of complex organisms made up of differentiated cells. As long as 'life' was synonymous with 'single cell', there was no need for apoptosis. What mattered was growth. It was life's nomadic era in which it was every cell for itself, and in which the hero's laurels went to those that overwhelmed their neighbors. But as cells accumulated more genetic information, could afford the luxury of differentiation, and assembled into ever more complex organisms, the cellular *laissez faire* of the olden days became dangerous. Cells still had to grow, of course, but now they also had to know when and where to stop, or even to disappear in order to make room for others. Life's new civilized era reserved the hero's laurels for the wise rather than the brave. Wise cells with their large genomes did not talk more, but knew how to say the right thing – or to say nothing – at the right time. Differentiation and embryonic development required the discipline to keep silent, and the art of stepping back.

The pitiless laws of evolution should never guide our own behavior. If they did, humans would stop being humane. Evolution cannot teach us ethics, but it can tell us much about complex organizations. Its business has been living matter, the most complex form of matter we know, and its business experience is close to 4000 million years. Has anyone heard of a more impressive proffesional resumè? For practical advice on how to run complex systems, evolution is hard to beat.

Reflecting on apoptosis can give us such practical advice. Most of us are no longer nomads, but we still cling to outmoded nomadic ideals. We adore the hero who goes it alone, be it with a sword, a six-shooter, or a laser gun. Such heroism will always have its place, but our fixation on it destabilizes our complex and differentiated societies. We should give the hero's laurels to the quiet heroism of *médecins sans frontières*, social workers, couples raising orphans, or altruistic political leaders. To those who can step back.

Knowing when to step back is also the hallmark of good teachers. Many of them try to shape students into a preconceived mold instead of letting them find their own way. Domineering teachers have damaged more students than overly permissive ones. Faculty members who constantly interrupt a student's seminar make me squirm, because watching over a talent calls for respect and patience. It needs the inner strength to step back.

Even science administrators might heed this advice. Managing science demands intelligence, organizational acumen, and scientific intuition, but also respect for the mystery of human creativity. Creativity is a delicate flower that wilts quickly when manipulated. Science policies that restrict research to 'relevant' questions kill creativity and are enemies of innovation. They forget that the more specific the question, the less surprising the answer.

My chemistry studies have earned me a doctorate in philosophy, and the 'phil.' after 'Dr.' strikes some as a little quaint. Yet I am very proud of this 'phil.'. Chemistry promised to explain many things about myself and the world around me, and chemistry has kept its word. But soon after my doctorate I stumbled across Erwin Schrödinger's little book 'What is life?' and was hooked. It seemed like the ultimate chemical question. I went into biology because I expected to learn about the mechanism of life. Now I also have learned about that of death. It comforts me that the two are so similar.

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