

D Y I N G



TO SEE



Studies of the lens of the eye not only could reveal ways to prevent cataracts but also might illuminate the biology of Alzheimer's, Parkinson's and other diseases in which cells commit suicide

By Ralf Dahm

RENEE LYNN Corbis [Impalas and Iionesses], JANA BRENNING [photocomposition]

AGING AND DAMAGE to the eye's lens can cause cataracts and yellowing that ruin vision, which may put the viewer at risk.

The lens

of the eye is the only transparent tissue in the human body. In the past few years, scientists have determined that this transparency—critical for focusing light—stems in large part from the unique ability of the lens to activate a self-destruct program in its cells that aborts just before completion, leaving empty but sustainable cells that transmit visible rays.

A better understanding of how lens cells become and remain transparent should suggest ways to prevent lens-clouding cataracts. More than half of all Americans older than 65 develop these sight-blocking occlusions. The only recourse is to surgically remove the person's lens and insert an artificial implant, and even then, complications requiring a second operation occur in a large proportion of patients. Given that cataracts affect primarily older people, for whom any kind of surgery is worrisome, a method to slow, stop or reverse cataracts would be a great aid indeed.

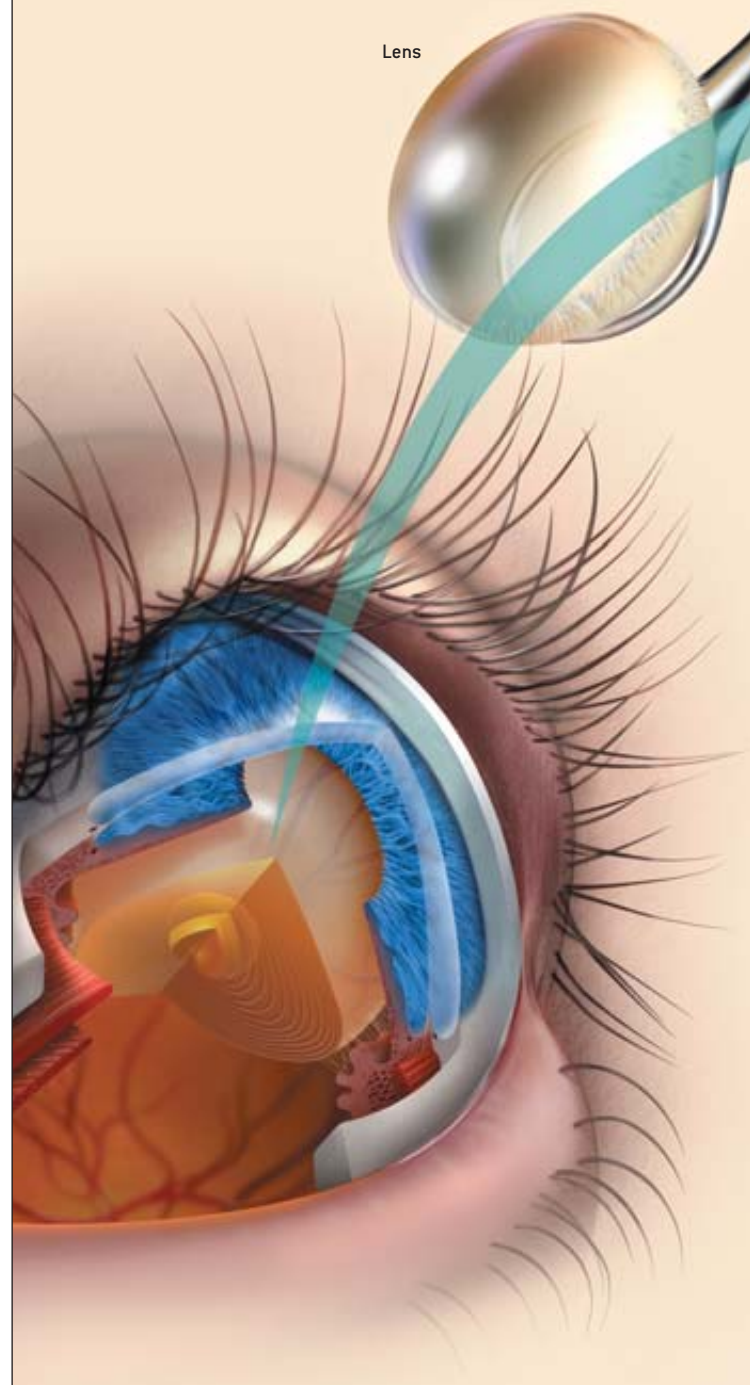
Beyond protecting vision, improved knowledge of how the lens tightly controls cell suicide could reveal ways to treat debilitating conditions characterized by excessive or inappropriate cell death, chief among them Parkinson's disease, Alzheimer's disease and chronic infections such as AIDS.

Barely Alive

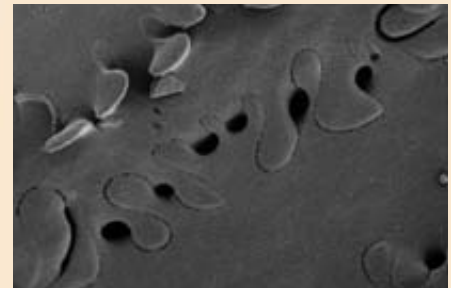
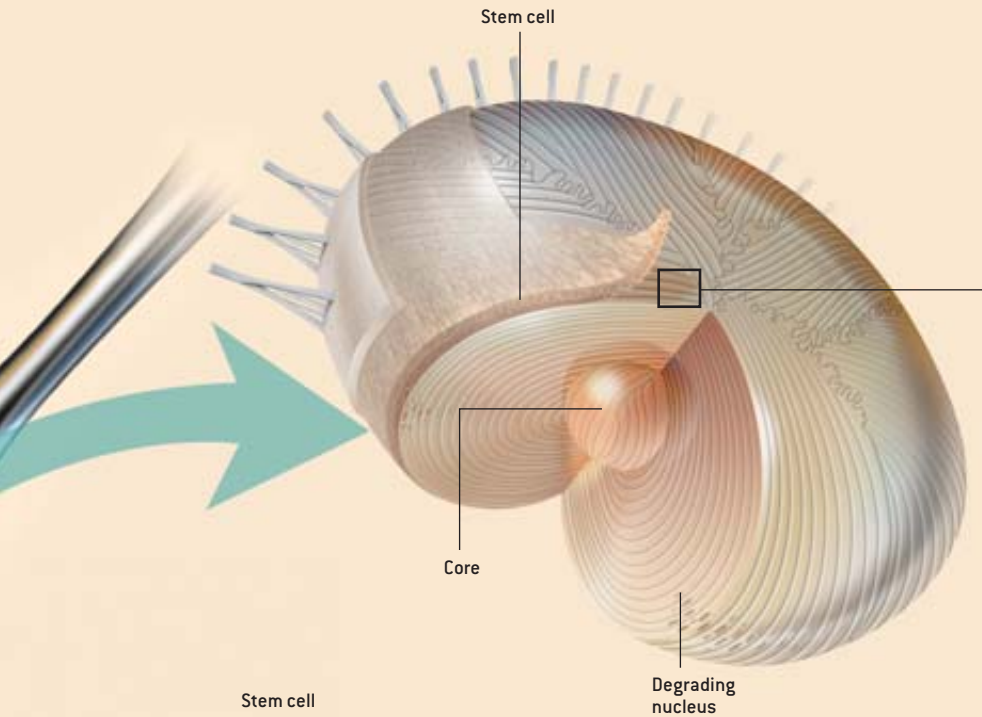
THE EYE'S LENS is a biological marvel, being at once dense, flexible and clear. If it bore the slightest obscurities, our visual world would be a fun house of warped and blurred images and glare. And if the lens had any hint of color, it would absorb light, preventing us from seeing certain shades.

Many animals possess translucent parts, such as insect wings, but truly transparent tissue in nature is rare and difficult to achieve. In humans the cornea is clear, but it is more a thin, gelatinous layer of proteins and sugars than true cellular tissue. The lens comprises about 1,000 layers of perfectly clear, living cells. Other than vision, the only significant exploitation of transparency

The eye lens is transparent both because of its architecture and because of its unusual developmental program. The cells of the fully formed lens fit together in a regular arrangement that limits the scattering of light (*diagram at right and micrographs at far right*). And those cells become free of light-obstructing material during development (*bottom right*) by initiating a suicide program that dissolves their innards but halts before the cells actually die.



THE LENS: KILLING ITSELF FOR CLARITY

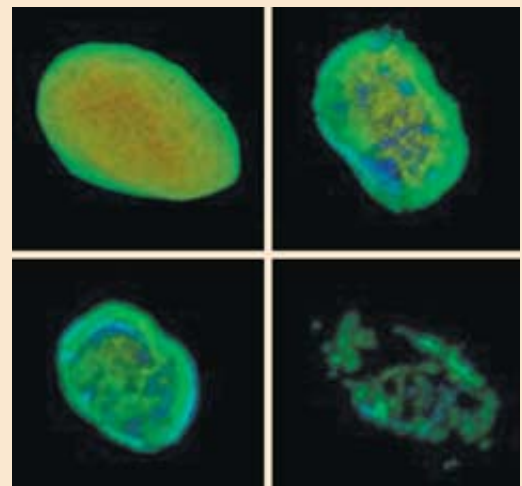
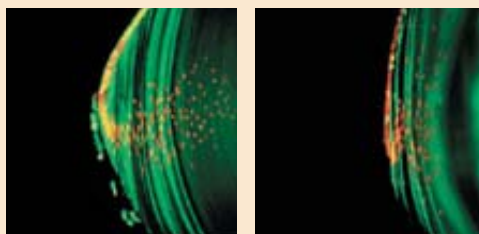


THE DEVELOPING LENS

Lens development begins in early embryos when undifferentiated (stem) cells lining a spherical vesicle (*left, top*) differentiate into lens cells that reach across the cavity (*left, bottom*). After this core forms, more stem cells differentiate into cells that elongate around the outside rim, adding layers in an onionlike manner (*above*). Initially these cells have a nucleus, mitochondria, endoplasmic reticulum and other typical organelles. But as they are encapsulated by newer cells, they degrade their organelles, leaving nothing but an outer membrane and a thick solution of special proteins called crystallins. This barely living material has a uniform index of refraction, so it does not scatter light.

Aspects of the process can be seen in a developing lens (*below, left*) and in a nearly fully developed lens (*below, right*) of a mouse. New cells stretch down across the equatorial region and effectively move inward as even newer cells cover them. Cell nuclei (*red*) traveling down and in persist for a time but dissolve as they are buried.

The nucleus of a developing lens cell dissolves over several days (*right*), with the nuclear envelope and DNA inside breaking down in tandem.



Layers of lens cells align in parallel (*top*), so that light passes perpendicularly through them, as in this bovine lens. Within a layer (*bottom*), adjacent cells interlock like jigsaw puzzle pieces to prevent gaps from forming; when the lens changes shape during focusing, the layering and interlocking of cells enables light to pass across cell boundaries without scattering.

KEITH KASNOT (drawings); RALF DAHM (top SEM, layers); ALAN R. PRESCOTT University of Dundee, Scotland (bottom left two images, nucleated cells); RALF DAHM (bottom right four images, degrading nuclei) IN THE LENS," BY V. I. SHESTOPALOV AND S. BASSNETT, IN JOURNAL OF CELL SCIENCE, VOL. 15, 2003

in the natural world occurs among certain ocean and freshwater creatures, which use the trait to blend into the open water and hide from predators. Yet almost all these animals, such as jellyfish, qualify only as “very translucent,” not totally see-through.

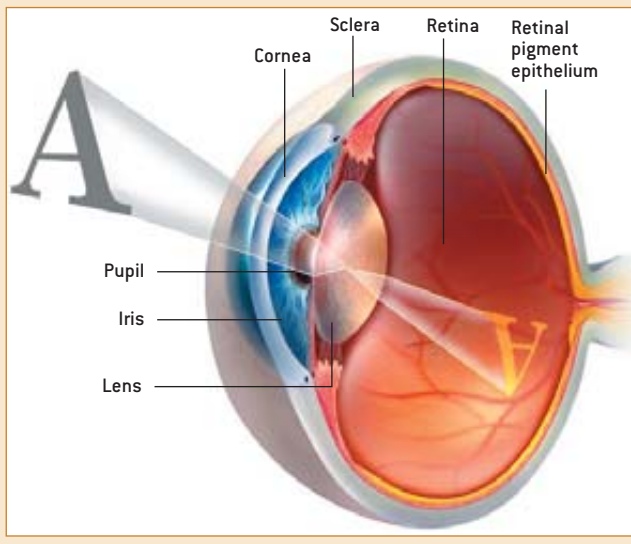
Transparency is unusual because cells have organelles—in-

Brown Eyes Blue

Knowing how the eye focuses light (*diagram*) explains not only how you see but why your eyes may be brown, hazel or blue—or red in a photograph. The iris blocks incoming light, leaving a neat hole—the pupil—through which light rays strike the lens and are focused onto the retina. The rays that hit the iris are scattered back. The shorter the light’s wavelength the greater the scattering, so blue light is scattered more than red, giving the iris a “natural” blue color. (The same principle causes the sky and sea to appear blue.) Yet the iris also contains melanin—pigment molecules that absorb various wavelengths. A lot of melanin will absorb much of the light, making the iris appear dark brown. Less melanin leads to lighter browns and greens, and very little melanin allows blue to dominate.

The pupil appears black because a melanin-rich layer of cells just behind the retina—the retinal pigment epithelium—absorbs all light that the retina has not. This absorption prevents light from randomly scattering back to the retina’s photoreceptors, which would blur vision. (The black lining of a camera serves the same purpose.) Because no light is emitted back through the pupil, it appears black.

Albinos cannot synthesize melanin; their retinal pigment epithelium does not absorb much light and thus causes poor vision and near-blindness in bright light. As light scatters back toward the pupil and iris, it illuminates blood vessels, making them appear pink or red. A similar effect can occur during flash photography of any person: the flash is so bright that the epithelium cannot absorb all the rays, and backscatter creates “red eye” in the photograph. —R.D.



ternal structures such as the nucleus (which stores DNA), the energy-producing mitochondria, and the Golgi apparatus and endoplasmic reticulum, which are important in the synthesis of proteins and lipids. Each structure has its own refractive index, and when a light ray crosses an area where the index changes, the light scatters, creating a degree of opacity.

In addition, some cells absorb certain wavelengths of light, resulting in color. The heme of the hemoglobin in blood cells gives them their characteristic red hue. Because organs and muscles have a blood supply, they appear primarily in shades of red, too. Furthermore, many cells, especially those in hair and skin, are populated with melanins—pigment molecules that come in colors ranging from red to black.

The lens has no melanins and no blood supply. Yet that alone is not enough for transparency. Cartilage has no melanins or blood supply and is colorless, but it is at best translucent. That is because in virtually all tissues, cells or fibers are oriented at various angles, creating different refractive indices that scatter light as it passes through. The lens is composed of only one cell type, and the cells are precisely aligned.

Given that lens cells have no blood supply, no connective or nervous tissue, and no organelles, can they even be considered alive? The answer depends on how “life” is defined. Lots of small animals without a blood supply are happily populating the planet. Human cartilage receives no blood, but any biologist would consider it living. If life means a cell has a metabolism, then lens cells are alive—albeit barely. Although they have no mitochondria to produce energy, certain nutrients and other molecules diffuse into the lens’s outermost cells and slowly pass inward, cell to cell.

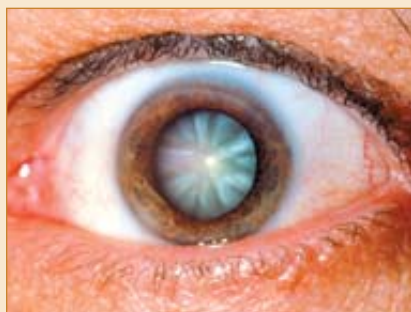
Young lens cells do have organelles when they first form from stem cells in a fetus, but the organelles are destroyed during early development. (The same occurs for new cells that are periodically laid down during adulthood.) What remains is a cytoplasm consisting of an unusually thick solution of special proteins called crystallins. Although the lens is often described as a crystal, it does not qualify in the chemical sense—where the geometric position of ions or molecules with respect to one another is systematically repeated. The lens is a “biological crystal”—that is, it has a very regular arrangement of cells. Each cell contains large molecules—crystallin proteins—that form complexes with paracrystalline arrangements. This construction makes the cytoplasm optically homogeneous; the refractive index does not change inside the cell or from one cell to another.

Seeing through a Glass, Dimly

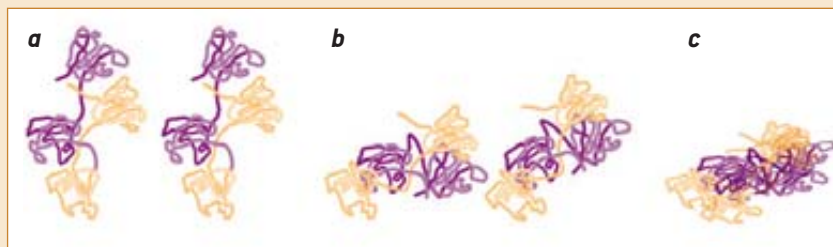
CLARITY, OF COURSE, comes at a cost. Although lens cells survive the controlled suicide of organelles, this degradation has drastic implications. Without nuclei, the genetic programs for synthesizing new parts are gone. Mature lens cells cannot regenerate or repair themselves, as cells in other tissues do.

The ability to replace damaged parts is a prime advantage of biological systems. The molecules that compose human cells typically have half-lives lasting a few minutes to several days. Within six months or so, 90 percent of the molecules that make

How Cataracts Form



Cataracts in the lens blur the vision or blind millions of people every year. Lens cells contain a thick solution of large proteins called crystallins (a) in an ordered arrangement.



Researchers are not yet sure why, but as crystallins accumulate damage, such as from ultraviolet light, oxidation or dehydration, they collapse into misfolded fibers (b). Then the misfolded proteins can aggregate into a tangled mass (c). The clumped mass blocks or distorts

incoming light, creating a cloudy spot in a person's field of view (photograph). Elevated levels of misfolded proteins have been found in the brains of people with Alzheimer's or Parkinson's disease, prompting scientists to look deeper for common clues. —R.D.

up our bodies are replaced by new ones. Lens cells, however, must function for a lifetime—a spectacular span.

This lack of repair mechanism makes the cells vulnerable to certain stresses. For example, severe dehydration can cause crystallin proteins to precipitate, prompting their cells to crumble into a clump—a cataract. This speck disrupts the otherwise uniform index of refraction, creating a cloudy spot in a person's field of vision. Just a few weeks of extreme dehydration can initiate cataract formation.

Even in the absence of such conditions, the inability to repair means that over the long term, small insults accumulate. Regular exposure to highly reactive molecules such as oxygen free radicals, or to ultraviolet radiation, or to years of elevated blood sugar from diabetes eventually leads to cataracts in many people—and to many cataract operations.

References to the removal of clouded lenses date back as early as 1800 B.C. to the Babylonian Code of Hammurabi. Ancient Egyptian texts and medieval European and Islamic writings describe detaching the lens from the ciliary muscle and pushing it down into the vitreous humor—the thick fluid in the back of the eye. Although this procedure removed the veil from the light path, it left no lens to focus rays. Patients could see only blurred images, as if their eyes were open underwater.

The application of special spectacles in the 17th and 18th centuries finally compensated for the lost focusing power. Today's artificial lenses eliminate any need for glasses. Doctors perform more than one million cataract operations annually in the U.S. alone. Fortunately, the procedure now has a success rate of nearly 100 percent and takes no more than 45 minutes. Still, approximately one third of patients return with after-cataracts, caused by undifferentiated cells—stem cells—that are inadvertently left behind during surgery. These cells start proliferating, but in contrast to their behavior during embryonic development, they form a disorganized mass that obscures vision and has to be surgically removed. In those developing

countries that lack surgical resources, cataracts account for half of all cases of blindness. In India alone, cataracts blind an estimated 3.8 million people every year.

In addition to becoming vulnerable to cataracts, the aging lens tends to yellow. Proteins that absorb blue and green light slowly accumulate, blocking these rays from reaching the retina and thereby giving the lens a yellow or brownish appearance. Only reds, yellows and browns pass through, altering a person's view of the world [see box on next page].

Controlled Suicide

IN RECENT YEARS, scientists have done much more than marvel at the lens's qualities and fret over its age-related decline. They are finding that the process by which the lens systematically destroys its organelles may offer a marvelous opportunity to solve some of humankind's most frustrating illnesses.

Like all cells, lens cells that arise from stem cells during early fetal development contain organelles. But as they differentiate, they demolish their organelles—and the rubble that remains—to become transparent. This may not seem problematic at first, but consider what happens when other cells encounter so much as a little damage to their DNA: they embark on an irreversible process called apoptosis, or pro-

THE AUTHOR

RALF DAHM is a project manager at the Max Planck Institute for Developmental Biology in Tübingen, Germany. He has a Ph.D. in biochemistry from the University of Dundee in Scotland and oversees a pan-European project to exploit zebra fish as a model for researching human development and diseases. He is co-editor of *Zebrafish: A Practical Approach* (Oxford University Press, 2002) and is author of a popular German-language science book about human embryonic development, stem cells and cloning. Dahm is also fascinated by how eye diseases change the way artists see and therefore render the world.

PAINTING THROUGH OLD EYES



CLAUDE MONET painted the Japanese bridge in his Giverny garden near Paris in 1899 (*left*). The same scene, which he attempted to capture again between 1918 and 1924, shows that cataracts had blurred his



vision and that the yellowing of his lenses had impaired his perception of blues and greens, leaving him in a world filled with murky reds and browns.

French impressionist Claude Monet (1840—1926) reached the grand old age of 86. But advancing years seriously affected his eyesight. Cataracts clouded his vision, and the yellowing of the lenses in his eyes altered his color perception. His work over the final two decades of his life offers a vivid depiction of how these common impairments skew human sight.

The yellowing started first. Gradually, proteins that absorb the “cold colors” of violet, blue and, later, green accumulated in his lenses, blocking these light rays from reaching the retina. Red and yellow light still passed through, rendering Monet’s world in increasingly warm tones.

Cataracts then clouded his vision, forcing him to perceive his surroundings

as if he were looking through frosted glass. Over time he had trouble discerning shapes, normal daylight became blinding, and in the late stages he could only differentiate between light and dark.

Monet first noticed that his eyes were changing during a trip to Venice in 1908. The 68-year-old painter had difficulty selecting his colors. In 1912 Monet’s doctor diagnosed a cataract in each eye and recommended surgery, but the artist was afraid; in his time any operation was fraught with problems, and removing a cataract frequently ended an artist’s career.

From that point on, however, Monet’s works show fewer details. Yellows, reds and browns predominate. When he

examined his later pictures, he was often seized by a towering rage and a desire to destroy them. In early 1922 he wrote that he was no longer able to create anything of beauty.

Later that year Monet’s right eye could only detect light and the direction from which it came; his left eye could only see about 10 percent of what is considered normal. In January 1923, at the age of 83, he finally had cataract surgery to his right eye, but he complained that the glasses he had to wear thereafter made colors appear peculiar.

In 1925 he finally found suitable spectacles and was delighted. He wrote that he could see well again and would work hard. Alas, he died a year later. —R.D.

grammed cell death. Destructive proteins released inside a cell chop up its DNA and key proteins, and the mitochondria shut down, depriving the cell of its energy source. The tattered cell breaks apart and dissolves. Ordinarily, damaged cells commit suicide to make room for new healthy cells—otherwise an organ with an accumulating number of damaged cells would not be able to function. In some cases, damaged cells kill themselves so they do not start proliferating and turn cancerous. Lens cells destroy the nucleus and every other organelle yet halt the process just before demolition is complete, leaving an intact outer membrane, an inner cytoskeleton of proteins and a thick crystallin plasma [see box on pages 84 and 85].

The ability to halt cellular suicide has come as quite a sur-

prise. The scientific community had always viewed apoptosis as an unstoppable process. Yet some unknown mechanism in the lens controls the death machinery so it destroys only certain cell components while leaving others intact. Several years ago I, along with other lens specialists, began to suspect that a deliberate braking mechanism was involved. We showed that specific compartments of differentiating cells—the nucleus or mitochondria, say—succumb to the same destruction that occurs during the full apoptosis of mature cells. But other compartments such as the cytoskeleton are unaffected. The implication is that lens cells actually use the death machinery not to destroy themselves but to choreograph the differentiation process.

The next leap in thinking came quickly: a mechanism that

could control apoptosis could alter the progression of diseases characterized by excessive cellular suicide, such as neurodegenerative disorders. To harness this power, researchers must find the signals—or blockers—that stop total destruction. Similarly, discovering what triggers lens cells to degrade their organelles could suggest new ways to induce cancer cells to commit suicide.

Pieces of evidence are accumulating. One theory advanced by Steven Bassnett of Washington University to explain the onset of apoptosis holds that during development, as new lens cells are formed around existing ones—like new layers around an onion core—the older internal cells become further removed from the surface, and the amount of oxygen that reaches them decreases. If the concentration drops below a threshold, the integrity of the mitochondria, which rely on an oxygen supply for energy production, might be compromised. Sensing this problem, the cell triggers the release of proapoptotic factors. This theory seems plausible in part because damaged mitochondria are known to initiate apoptosis in mature human cells. The death machinery is always there, ready to go. If the cell senses serious damage, it can release the block on the death machinery, and all hell breaks loose.

At the same time, Bassnett has proposed another potential cause of apoptosis: the lactic acid produced during the breakdown of glucose that occurs in differentiated lens cells. Mature cells in the lens's center lack mitochondria and produce energy by turning glucose into lactic acid. The acid forms a concentration gradient, along with a gradient in pH. Either gradient could start apoptosis.

Other triggers have attracted attention as well. In studies of lens cells in culture, Michael Wride, now at Cardiff University in Wales, and Esmond Sanders of the University of Alberta in Canada showed that tumor necrosis factor appears to promote the degradation of lens nuclei. Tumor necrosis factor is a messenger protein, or cytokine, that can act as a potent inducer of apoptosis in healthy cells and certain tumor cells. No one knows how this cytokine would work naturally in the lens, however.

Klaus van Leyen of Massachusetts General Hospital and his colleagues have uncovered clues to the molecules that respond to cell-death triggers. They found, for instance, that the enzyme 15-lipoxygenase can embed itself in the membranes of lens cell organelles and create holes in them. The holes allow proteases (enzymes that destroy proteins) to enter and destroy the organelles. Exactly what elicits the 15-lipoxygenase activity at the right time during lens cell differentiation remains unclear.

Research by this author and others has recently provided possible insights into the braking mechanism. In human, rat and mouse lenses, my colleagues and I found that a protein called galectin-3, which can bind to other molecules, is produced in lens cells that still have their organelles, but its synthesis is reduced when the organelles start to degrade. This activity pattern could control the apoptosis process, but we have no idea what triggers the shutoff of galectin-3. We began to look at galectin-3 because it is known to be involved in vari-

ous biological functions related to cell proliferation, apoptosis and differentiation in other tissues.


Most recently, Sogo Nishimoto of Osaka University in Japan has identified a DNase (an enzyme that cleaves DNA) that is essential for the degradation of DNA in lens cells. When this particular DNase is missing in laboratory mice, they are born with cataracts; also, the apoptotic breakdown of the nuclei during the differentiation of lens cells does not seem to occur, whereas apoptosis appears to occur normally in all other cells. (Children can be born with cataracts if organelles are not degraded during fetal development, possibly as a result of a viral infection, such as rubella, in the mother.)

Of course, it is conceivable that rather than actively halting apoptosis in midstream, lens cells forestall death because some components simply are resistant to the molecules that effect self-destruction. For instance, proteins occurring only in the lens might be “invisible” to the killer enzymes that degrade the cytoskeletons of other cells. Alternatively, some evidence suggests that crystallins might form a protective barrier around certain proteins, preventing the enzymes from reaching those targets.

Swimming Zebras

AS WORK ADVANCES, a small fish could offer promising clues. The zebra fish is a terrific creature in which to study embryonic development. Its embryos have very few cells and are quite translucent early on, so experts can observe the formation of internal organs. Most organs develop incredibly fast—just 48 hours after eggs are laid. During day three, the fish hatch and start swimming around. Yet because zebra fish are vertebrates, the genetic control of their development is remarkably similar to that in humans.

Various groups have undertaken large-scale searches for mutant zebra fish, among them the laboratory of Nobel laureate Christiane Nüsslein-Volhard at the Max Planck Institute. Among the mutants found were ones possessing lenses with intact organelles and others with lens cells that died completely. Some mutants had cataracts much like those in humans.

The labs are now looking to see if these mutants can provide new information about what starts and stops apoptosis. If so, the insights could advance medical research into ways to beat cell-death diseases. In the meantime, these studies should greatly improve our understanding of how and why cataracts form, which could lead to ways to slow their growth or prevent them altogether. That possibility alone keeps us focused. 

MORE TO EXPLORE

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