of the inner core requires a heat flow of ~15 TW. About 40% of this heat flow is carried by conduction across the core, leaving only 60% to contribute to fluid motions and the maintenance of the magnetic field.

Changes in the heat flow with time are controlled mainly by changes in the thickness of the thermal boundary layer at the base of the mantle, because changes in the temperature across the layer are comparatively small. It is possible to increase the heat flow to 15 TW prior to 1000 million years ago with plausible models for the effects of temperature-dependent viscosity, but the resulting thermal histories invariably produce unrealistically high temperatures in the core by 2000 to 3000 million years ago. So how can we reconcile the high heat flow needed to maintain Earth's magnetic field with reasonable thermal histories at early times?

There appear to be two ways out of our present difficulties. The first (and perhaps less likely) option is that the power requirements for the magnetic field are much less than 1 TW. With a heat flow sufficient for maintaining a power requirement of just 0.1 TW, we can extend the age of the solid inner core to 3600 million years and avoid high early temperatures (*11*). However, the present-day heat flow would then have to be substantially lower than 6 to 12 TW. This discrepancy could be explained by accumulating radioactive elements at the base of the mantle, because such additional heat sources decrease the temperature gradient, and hence the heat flow, at the core-mantle boundary. Such an enriched layer could be formed by fractionating radioactive elements into a dense silicate melt at the base of the mantle. A thin zone of partial melt is often invoked to explain anomalously low seismic velocities in this region (*12*). An alternative source of radioactive material may be a continuous supply of subducted oceanic crust to the core-mantle boundary (*13*).

A second option requires additional heat sources in the core itself. These heat sources would slow the cooling of the core for a prescribed heat flow and therefore extend the age of the inner core. $^{40}$K is most commonly cited as a potential radiogenic heat source in the core (*14*), although there is little experimental evidence for partitioning large amounts of K into liquid iron (*15*) during the formation of the core. However, a present-day concentration of 200 ppm K (by mass) is sufficient to avoid high temperatures at early times.

My thermal history calculations show that 200 ppm K has only a small influence on the age of the inner core (increasing from 950 to 1300 million years for a heat flow of 9 TW). But the effect on the thermal history at earlier times is dramatic because $^{40}$K has a short half-life, causing a substantial increase in heat production in the past. A K concentration of 400 ppm could plausibly cause the core to warm at early times before cooling to its present state. The inner core (if present) would shrink during the initial warming and subsequently grow to its current size during cooling.

Advances in experiments and theory have come a long way in our pursuit of Birch's challenge to determine the thermal state of the core. Yet, the thermal history that brought us to this state remains unclear. Better knowledge of the partitioning of all radiogenic elements between various reservoirs in the planet will help to reduce some of the ambiguity. More reliable estimates of the power requirements for Earth's magnetic field would place tighter bounds on the allowable heat flow. Progress on these issues will undoubtedly raise new questions if the past is any guide.

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CELL BIOLOGY

# A Matter of Life or Death

**Rama Ranganathan**

Photoreceptor cells of both the vertebrate and invertebrate retina have remarkable functional properties that mediate vision. These cells reliably detect single photons under dim light conditions and yet continuously respond to a wide range of light intensities through a finely tuned phototransduction signaling cascade. Rhodopsin, the light-sensitive pigment in photoreceptor cells, is arguably the best understood member of the diverse G protein–coupled receptor (GPCR) family. Visual phototransduction has served as a model for many other G protein signaling systems. Recent work, including the study by Acharya *et al.* (*1*) on page 1740 of this issue, reveals new molecular connections between the phototransduction cascade and another crucial cellular signaling process, programmed cell death (apoptosis).

Diseases of retinal degeneration, such as retinitis pigmentosa, age-related macular degeneration, cone dystrophy, and Oguchi's disease, are all associated with mutations in different components of the visual signaling cascade (*2, 3*). Despite the diversity of molecular sites affected, these mutations all share a common final outcome: the apoptotic death of photoreceptor cells (*3*). The compound eye of the fruit fly *Drosophila* has proved to be a facile model system for dissecting the molecular mechanisms of photoreceptor cell death. Genetic screens that score characteristic changes in eye morphology due to photoreceptor cell loss identify two classes of retinal degeneration mutants. The first class comprises mutations that induce the light-independent death of retinal cells. These mutations are typically found in genes encoding structural components of the rhabdomere (the tightly packed set of microvillar membranes that houses the visual signaling machinery) or genes required for the synthesis of rhodopsin (*4*). The second class comprises mutations that cause light-dependent photoreceptor cell death and involve mutations in genes encoding several key components of the visual signaling cascade.

The second class of fly eye mutants can be further subdivided according to their requirement for productive visual signaling. For example, *arr2* mutants expressing low amounts of the protein arrestin (which shuts off activated rhodopsin) display light-dependent necrotic death of photoreceptor cells, which is suppressed by deleting the Gα protein required for visual signaling (*5*). A similar form of retinal degeneration is found in *rdgA* mutants carrying

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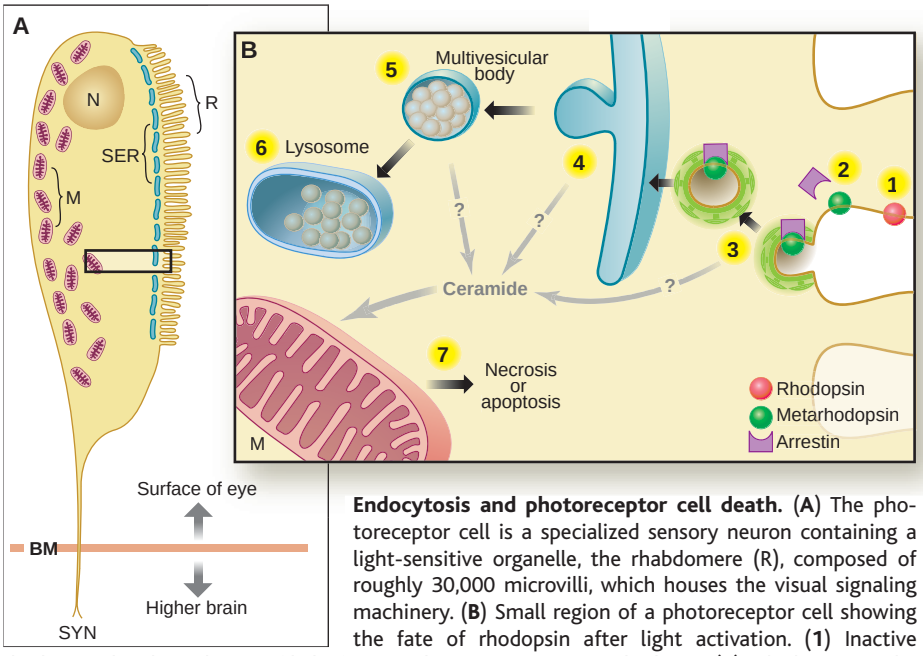


**Endocytosis and photoreceptor cell death.** (**A**) The photoreceptor cell is a specialized sensory neuron containing a light-sensitive organelle, the rhabdomere (R), composed of roughly 30,000 microvilli, which houses the visual signaling machinery. (**B**) Small region of a photoreceptor cell showing the fate of rhodopsin after light activation. (**1**) Inactive rhodopsin absorbs a photon of light forming the active state, metarhodopsin (**2**), which triggers the visual signaling cascade (not shown). Metarhodopsin binds to arrestin and becomes internalized through a clathrin-dependent process (**3**). Internalized vesicles first arrive at an early endosomal compartment (**4**) and then proceed to a stable internal compartment, the multivesicular body (**5**). Presumably, the terminal step is the fusion of the multivesicular body with lysosomes (**6**). Fly mutants defective in some visual signaling components, such as arrestin, display light-dependent necrosis of photoreceptor cells. In contrast, fly mutants defective in other signaling components, such as phospholipase C–β, show apoptosis of photoreceptor cells. Both forms of photoreceptor cell death involve accumulation of ceramide (*1*). M, mitochondria; N, nucleus; SER, smooth endoplasmic reticulum; BM, basement membrane; SYN, synapse.

loss-of-function mutations in a diacylglycerol kinase required for termination of the phototransduction cascade (*6*), or in $trp^{P365}$ mutants with mutations that constitutively activate the light-gated calcium-permeable ion channel Trp (*7*). In contrast, some signaling mutants show retinal degeneration that requires light activation of rhodopsin but does not require downstream signaling through G proteins. An example is the *norpA* mutant that lacks the phospholipase C component of the visual signaling pathway. These mutant flies are blind but paradoxically display photoreceptor cell death upon light stimulation (*8*). These light-dependent but G protein–independent forms of retinal degeneration culminate in the apoptotic (rather than necrotic) death of photoreceptor cells (*5, 8*).

How does activation of rhodopsin lead to apoptosis of photoreceptor cells in the absence of visual signaling? It turns out that arrestin molecules in *Drosophila* photoreceptors mediate clathrin-dependent internalization of activated rhodopsin (metarhodopsin) into a stable intracellular compartment (see the figure) (*5, 8*). Interestingly, photoreceptor cell apoptosis requires the internalization and long-term sequestration of phosphorylated metarhodopsin–arrestin complexes (*5*). However, it is not yet clear how the internalization of signaling components is connected to triggering of the cell death machinery. A central objective continues to be the identification of other molecules that regulate the survival of retinal photoreceptor cells.

With this goal in mind, Acharya *et al.* (*1*) now report that ceramide, a sphingolipid involved in signaling, controls the viability of fly photoreceptor cells. Specifically, these investigators show that photoreceptor cell death in both hypomorphic $arr2^3$ mutants (which degenerate through necrosis) and $norpA^{P41}$ mutants (which degenerate through apoptosis) can be rescued by engineering these cells to express ceramidase, an enzyme that hydrolyzes ceramide to sphingosine. Flies produce ceramide in two ways: through the breakdown of ceramide phosphoethanolamine (analogous to sphingomyelin in mammals) or through de novo synthesis from palmitate (*9*). Acharya *et al.* (*1*) also report survival of $arr2^3$ photoreceptor cells in mutants defective in the rate-limiting enzyme of the de novo ceramide synthetic pathway, consistent with the notion that an increase in ceramide promotes photoreceptor cell death. Accumulation of ceramide has been implicated in the early phase of the apoptotic response in diverse cell types subjected to a variety of insults, such as oxidative stress (*10*).

How does ceramide participate in cell death decisions? Two experiments by Acharya *et al.* (*1*) provide important initial clues. First, the authors show that expression of ceramidase rescues photoreceptor cell degeneration in $arr2^3$ mutants without altering the slowed inactivation of the light response resulting from defective metarhodopsin shutoff. Thus, signaling events other than the increased flux of calcium ions due to sustained Trp channel activity must be required to trigger cell death. Second, because $arr2^3$ mutants are also defective in clathrin-mediated endocytosis of metarhodopsin, the authors tested the possibility that other mutants defective in endocytosis similarly control ceramide-dependent photoreceptor cell survival. They discovered that a dominant negative mutation in the *shibere* ($shi^{ts1}$) gene—which encodes the dynamin guanosine triphosphatase required for clathrin-dependent endocytosis (*11*)—causes retinal degeneration. This degeneration could be suppressed by inducing photoreceptor cells to express ceramidase.

The Acharya *et al*. data provide two new insights: (i) Clathrin-mediated internalization of rhodopsin is essential for photoreceptor cell survival, and (ii) ceramide production is tightly coupled to the internalization process. Signaling via sphingolipids is required for endocytosis in yeast (*12*), and ceramide regulates phagocytosis by COS-1 cells (*13*). It will be interesting to see whether similar signaling processes could explain the interaction between ceramide metabolism and endocytosis in fly photoreceptor cells.

It is particularly intriguing that ceramidase expression rescues photoreceptor cells from both necrosis and apoptosis. This seems surprising at first glance. Apoptosis is a well-regulated death process in which cell disassembly proceeds in an orderly fashion with an intact plasma membrane. In contrast, necrosis is an unregulated pathological process that ends in plasma membrane rupture and leakage of cellular contents (*14*). However, recent studies suggest that these two outcomes are really distinct endpoints of the same molecular process: mitochondrial injury by components such as ceramide resulting in opening of the permeability transition (PT) pore in the inner mitochondrial membrane (*14*). Opening of the PT pore causes massive cellular changes that drive both necrosis (for example, through uncoupling of oxidative phosphorylation, release of $Ca^{2+}$ ions, or depletion of cellular redox potentials) and apoptosis (through release of proapoptotic factors such as cy-

tochrome c). In this model, deciding between necrosis and apoptosis depends on two events: the cellular stores of adenosine triphosphate at the time of PT pore opening, and competition between the rate of bioenergetic collapse and activation of the apoptosis cascade. Clearly, a principal goal is to understand the mechanisms that tip photoreceptor cells in favor of either necrosis or apoptosis in response to cellular stress. Diverse *Drosophila* mutants with a variety of visual system defects promoting either necrosis or apoptosis in response to light activation are important reagents for the systematic dissection of this decision process. The Acharya *et al*. work points the way by identifying ceramide as the potential integrator of the different pathways leading to photoreceptor cell death.

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CANCER

# Developing Molecular Biomarkers for Cancer

**David F. Ransohoff**

The development of a noninvasive test for cancer has been the Holy Grail of cancer detection research for three decades. In 2003, there is reason to be cautiously optimistic that such a test can actually be developed. On page 1753 of this week's issue, Cui *et al*. (*1*) report a step forward in this endeavor. They have developed a DNA-based blood test that may predict the risk of developing colorectal cancer (CRC). Their work raises interesting issues about cancer biology, the use of biomarkers to test for cancer, and the process of biomarker discovery.

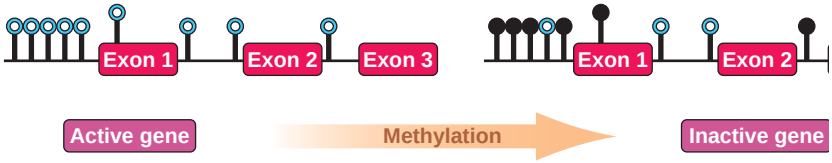
Cui *et al*. examined colon tissue biopsies and blood samples from 172 colonoscopy patients for loss of imprinting (LOI) in the insulin-like growth factor II (*IGF2*) gene. LOI is an epigenetic phenomenon in which certain genes are generally silenced during embryonic development through the addition of methyl groups (methylation) (see the figure). Gene methylation also may take place later in life. Cui and colleagues show that LOI of the *IGF2* gene is associated with a family history of developing CRC and with a personal history of colon adenomas and CRC (*1*). The association of LOI with familial CRC is potentially important because 30 to 50% of sporadic CRC is associated with familial risk, yet the genetic basis for this common association is largely unknown. In contrast, genetic mechanisms for the dramatic but very uncommon inherited CRC syndromes—familial adenomatous polyposis coli and hereditary nonpolyposis colorectal cancer—are well established.

These investigators demonstrated that LOI was present in 28% of persons with a family history of CRC and—although the number of patients was small—in 56% of those with a personal history of CRC. In contrast, LOI was present in only about 10% of healthy individuals. If LOI turns out to "mediate" risk for common sporadic CRC, this relationship could provide not only the basis for a blood-based diagnostic biomarker but also insights into the biology of sporadic CRC.

LOI may work through the epigenetic phenomenon of methylation. Both DNA methylation and DNA mutations disrupt the transfer of information from DNA to RNA to the functional protein. Methylation of cytosines in the DNA is often associated with changes in gene expression. In many cases, methylation results in repression of transcription (*2*) (see the figure). The regulation of methylation is complicated and interesting because, unlike mutation of the DNA, methylation can be chemically reversed, and it can be either acquired or inherited. The product of the *IGF2* gene is an important tumor growth factor, and the DNA sequence that regulates expression of *IGF2* is normally switched off through methylation. However, with LOI the methylation status of this DNA sequence is reversed. In the subjects studied by Cui *et al*., LOI appears to have been inherited or acquired early in life because it turned up in multiple biopsies of colon tissue, and sometimes in white blood cells as well. In contrast, LOI would be expected to be patchy in colon tissue if it occurred later in life from clonal expansion of cells at a single focus.

The work of Cui *et al*. is a step toward the goal of developing a noninvasive test for detecting cancer, but whether it will be useful clinically requires consideration of several factors. First, LOI does not assess directly the presence of a CRC, but rather the "tendency" of colon tissue to become cancerous. A marker as indirect as one that assesses "tendency" might nevertheless be clinically useful if it, either alone or with a panel of other markers, were sufficiently sensitive that a negative test strongly predicted a low lifetime risk of CRC, making conventional screening unnecessary (*3*). Identification of a large subgroup of persons for whom conventional screening including colonoscopy is unnecessary would constitute a major advance in the development of cancer biomarkers.

Other types of molecular marker research may be aimed not at measuring the lifetime "tendency" of developing adenoma or CRC but rather at direct detection of




**Switching genes off.** Methylation of cytosine bases in DNA prevents the DNA from being read, resulting in silencing of the gene. Methylation can be both inherited or acquired and is potentially reversible.

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