Retinitis pigmentosa: cone photoreceptors starving to death

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In retinitis pigmentosa, rod and cone photoreceptors die. Although rods die as a consequence of rod-specific genetic mutations, there is no clear explanation for the progressive loss of cones. A new study in this issue suggests that changes in the insulin/mTOR pathway and cell starvation can partially account for cone death in this disease.

Every year, 1.5 million people worldwide lose their sight as a result of a retinal degeneration disorder known as retinitis pigmentosa. Retinitis pigmentosa is a collection of inherited disorders caused by mutations in a wide variety of genes that are mostly expressed by rod cells, the photoreceptors that are responsible for night vision[1]. In theory, loss of rod function should not pose a severe problem in many urban areas, as, even at night, illumination is sufficient to activate cones, the photoreceptors that are responsible for daylight vision[1]. For millions of individuals with retinitis pigmentosa throughout the world, however, this is of little consolation: they eventually become completely blind because their cones die progressively, even though the cones do not express a mutated protein. In this issue, Punzo and colleagues[2] take advantage of various mouse models of retinitis pigmentosa to address the mechanism of delayed cone death in rod dystrophies. Their results indicate that it may be possible to treat cones by stimulating their metabolism by activating the insulin/mammalian target of the rapamycin (mTOR) pathway.

The rod to cone ratio in the mouse retina is similar to that of the human retina. This makes it a good model for studying human retinitis pigmentosa. Moreover, there are several mouse lines that mimic different forms of human retinal degeneration[3]. Punzo and colleagues[2] searched for common cellular and molecular patterns at the onset of cone degeneration in four different retinitis pigmentosa mouse lines harboring mutations in rod-specific genes (*Pde6b*$^{-/-}$, *Pde6g*$^{-/-}$, *Rho*$^{-/-}$ and P23H[3,4]), assuming that the mechanism of progressive cone death would be the same in all rod dystrophies. Despite the genetic heterogeneity associated with the disease, rod photoreceptors always die by apoptosis, although the kinetics of rod death is quite distinct across the four models. Similar to human retinitis pigmentosa progression, Punzo *et al.*[2] found that cone degeneration in these mice showed common temporal landmarks; it always started with

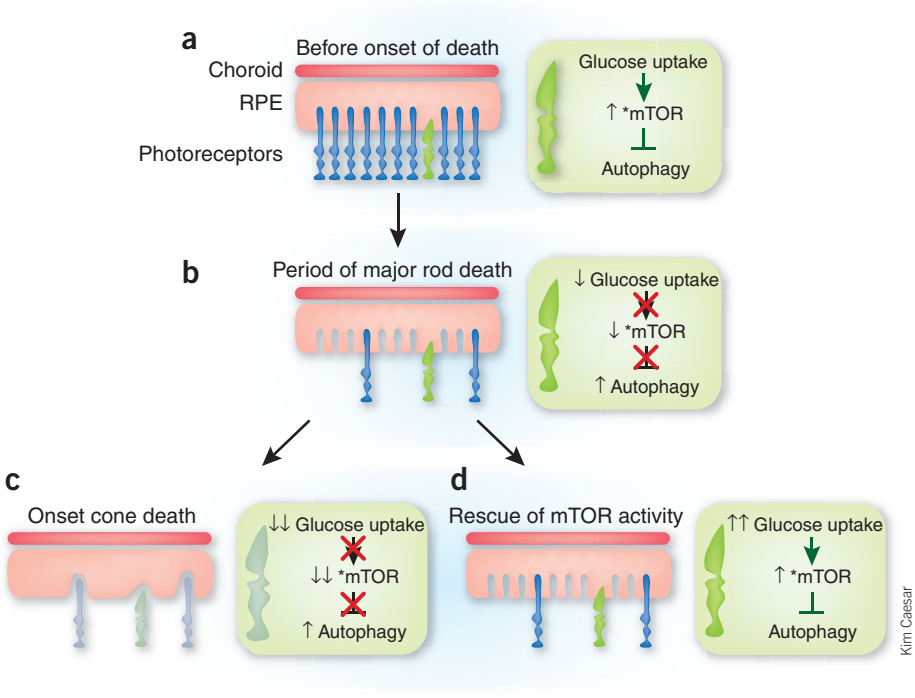

Figure 1 Alteration in the insulin/mTOR pathway modulates non–cell autonomous cone degeneration in retinitis pigmentosa. (**a**) In a healthy retina, the contacts between the RPE and the rod and cone photoreceptors assure appropriate uptake of nutrients that activate mTOR (*mTOR) and inhibit autophagy. (**b**) In retinitis pigmentosa, rod loss triggers metabolic changes in the cones. (**c**) Perhaps as a consequence of diminished RPE-photoreceptor interaction, glucose uptake is compromised, active mTOR levels decrease, cone outer segment shortens, CMA is initiated and cones finally die. (**d**) Insulin treatment recovers mTOR activity and rescues cones from death.

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a central-to-peripheral progression after the major phase of rod death, its duration was always proportional to that of rods' degeneration and was preceded by a reduction in the length of the cone outer segment.

Previous studies have suggested that non–cell autonomous cone death may be explained by deprivation of rod-derived trophic factor(s), environmental alterations associated with rod apoptosis (such as liberation of toxins), loss of rod-cone gap junctions, microglia mobilization or oxidative stress[5,6]. However, the possibility that these events cause retinal degeneration is questioned by the late onset and slowness of cone death relative to that of rods. Furthermore, in a few retinitis pigmentosa cases in which cone-specific genes are mutated, cones die, but rods survive. This suggests that general tissue reactions caused by cell death are not sufficient to trigger photoreceptor loss. Instead, massive rod loss probably causes changes that specifically make the cones vulnerable over time. Exploiting their morphological data, Punzo *et al.*[2] established criteria to perform a comparative analysis of the retinal transcriptome by selecting and enriching mRNA pools during equivalent time points of cone degeneration in the four mouse models. Genes involved in metabolism, in particular those belonging to the insulin/mTOR signaling pathway, were identified as being the most affected.

mTOR is a serine-threonine kinase that integrates intracellular responses to trophic factors, mitogens, hormones, stress, nutrients and the energy status of the cell. Under normal conditions, activated mTOR phosphorylates a large variety of targets that favor homeostatic events, such as protein translation, and inhibits others. Under stress conditions such as diminished nutrient availability or hypoxia, mTOR shifts the balance toward the opposite direction[7]. Consistent with the microarray data, Punzo and collegues[2] observed that activated mTOR, localized mostly in the dorsal retina, was progressively reduced in the four mouse models analyzed and coincided with the time course of cone death. Glucose depletion caused a similar decrease in activated mTOR in cultured wild-type retinas, suggesting that reduced levels of active mTOR in the mutant retinas may reflect poor nutrient conditions. If this were the case, cones should show characteristics of cells trying to overcome nutrient deprivation across the four mouse models. Indeed, Punzo *et al.*[2] found that degenerating cones consistently showed high levels of hypoxia-inducible factor 1 (HIF-1) and its direct target, glucose transporter 1. HIF-1 is a transcription factor that improves glycolysis in stressed conditions and its expression level is inversely proportional to mTOR activity[7].

In cells with restricted nutrient conditions, low mTOR activity also rapidly activates the process of autophagy, whereby intracellular proteins and organelles are reabsorbed in vacuole-like structures to retrieve cellular nutrients. There are two types of autophagy: macroautophagy and chaperone-mediated autophagy (CMA). Macroautophagy is generally triggered by acute short-term nutrient deprivation. CMA, however, is activated under conditions of prolonged nutrient depletion or oxidative stress and is characterized by increased expression of the lysosome-associated membrane protein 2A (LAMP-2A). Punzo *et al.*[2] could not find clear evidence for increased macroautophagy but did observe notable expression of LAMP-2A specifically in the degenerating cones. Thus, in the absence of rods, cones die from starvation with a mechanism that probably involves compromised glucose uptake and low mTOR activity (**Fig. 1a–c**).

Insulin controls glucose uptake in cells and activates mTOR function[7]. As a proof of principle, Punzo and colleagues[2] went a step further and tested whether administration of insulin could rescue cone degeneration. Although limited, the results are encouraging, as daily systemic administration of insulin maintained the levels of active mTOR and improved cone survival in the *Pde6b*$^{-/-}$ model over a 4-week period. Prolonged administration of insulin causes receptor desensitization and subsequent increase of blood glucose levels. The drug streptozocin causes a similar increase in glycemia by killing insulin-producing cells in the pancreas. Punzo *et al.*[2] found that cone degeneration was accelerated in *Pde6b*$^{-/-}$ mice that were treated with streptozocin. This result suggests that the promotion of glucose uptake by insulin, but not the hyperglycemia itself, ameliorates cone survival.

Overall, this work provides a new mechanism for explaining cone death in retinitis pigmentosa, but a major question remains. Why do cones become nutritionally deprived in the absence of rods? Cone death starts when rods are reduced to a single row of cells. Therefore, Punzo *el al.*[2] proposed that decreased cell density might be the reason behind the progressive and delayed nature of cone degeneration. Normally, the retina pigmented epithelium (RPE), located at the interface between the photoreceptors and the vascular meshwork of the choroid layer, provides nutritional support and oxygen to the retina and phagocytes the outer segment of the photoreceptors, assuring its renewal[8]. This exchange is made possible by a large number of contacts that are established mainly by the RPE and the outer segments of the rods, as this cell type accounts for 95% of the total photoreceptors in mammals. Massive rod loss may drop the RPE-cone outer segment interactions under a critical level, compromising the uptake of nutrients. In zebrafish, where the overall ratio of cones versus rods is reversed, a mutation in a cone-specific phosphodiesterase leads to cone degeneration that is associated with a secondary rod loss in regions of low rod concentration, supporting the idea that secondary photoreceptor death may relate to cell density[9].

In the human retina, lost photoreceptors are no longer replaced. Although replacement of the missing or defective gene in rods may seem to be the most straightforward approach to retinitis pigmentosa therapy, the large number of genes associated with the disease makes the effort challenging. Research efforts have thus focused on rod replacements by injecting either engineered stem cells[10] or immature postmitotic rods[11] into the subretinal space of different retinitis pigmentosa mouse models. This last strategy has raised many expectations, as immature postmitotic rods integrate successfully into the retina and establish synaptic contacts, restoring the pupillary light reflex[11]. Unfortunately, as it currently stands, the level of integration is insufficient to restore visual acuity.

Therapies directed to preserve the cones that remain alive may thus be a very effective alternative for improving the quality of life of many retinitis pigmentosa patients. One strategy is to supply trophic/protective factors to the cones, and a promising candidate molecule is the rod-derived cone viability factor[12]. Its gene encodes a long isoform with a putative thioloxydoreductase activity that may protect from oxidative stress and a short form with trophic activity for cones[5,13]. Although tempting to speculate given the initial results of Punzo *et al.*[2], using insulin as an effective treatment for retinitis pigmentosa patients is questionable. Prolonged insulin administration did not further improve cone survival and there are adverse effects of systemic administration of this hormone. Nevertheless, the work of Punzo *et al.*[2] points to the importance of maintaining active mTOR levels, which could be the target of future and more specific therapeutic approaches directed to rescue cones from inevitable death (**Fig. 1d**). Besides favoring nutrient uptake, an mTOR-directed therapy will have the additional advantage of regulating the translation of proteins that are directly related to apoptosis and cell survival. It may also restore cone opsin levels[7] and possibly maintain structural proteins involved in RPE-photoreceptor contacts, which may perhaps further aid cone survival. For a monogenic

disease with genetic heterogeneity such as retinitis pigmentosa, attacking a common downstream pathway leading to photoreceptor degeneration, rather than targeting numerous defective molecules, would be a sensible avenue toward a universal treatment. The results of Punzo *et al.*[2] give us new promise in proposing such plausible targets.

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Sniffing out a function for prion proteins

Donald A Wilson & Ralph A Nixon

When prion proteins go wrong, they can do serious damage, but little is known about their normal function, despite their ubiquitous expression in the brain. A new report in this issue suggests a critical role for prions in olfactory discrimination.

Although the word prion was coined by Stanley Prusiner to describe the "proteinaceous infectious particle" that causes a family of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies over 20 years ago, little is known about the normal function of prion proteins. Most of what is known about them comes from studies of their involvement in these devastating diseases, which include Creutzfeld-Jakob disease, bovine spongiform encephalopathy ('mad-cow disease') and chronic wasting disease in elk and deer. These diseases are distinguished by rapidly progressive neurological deterioration and a pattern of neurodegeneration that is characterized by prominent vacuolization of neuronal cytoplasm, which gives the brain a sponge-like histological appearance. The key pathogenic event in these diseases is the conversion of an endogenous cell-surface glycoprotein, the prion protein (PrP^{c}), to a pathological isoform (PrP^{sc}) that has an abnormal conformation and an unusual resistance to proteolytic degradation (**Fig. 1**). PrP^{sc} accumulates in cells and plaque-like extracellular deposits, converting more PrP^{c} into the pathogenic form and triggering neurodegeneration by mechanisms that are still not fully understood. Conversion of PrP^{c} can be a result of inherited mutations, infection of the host with a prion-infected tissue or rare sporadic events[1]. Although the formation of PrP^{sc} is believed to result in a gain of toxic function, a loss of function of PrP^{c} has not been excluded as being involved in prion disease

PrP^{c} is most abundantly expressed in the brain and it would be expected that the loss of this protein would result in substantial neurobehavioral modifications. However, the specific role of PrP^{c} in neural function and behavior is far from clear[2]. In fact, previou. work suggests that the most robust phenotype of PrP^{c} loss in transgenic mice is protection from prion diseases[3]. Although changes in PrP^{c} expression influence a variety of critical cellular processes in neurons, including cell survival, synaptic maintenance and plasticity, and axonal maintenance[2], data on these issues have occasionally been contradictory. Thus, 'elusive' remains one of the descriptors most commonly attached to this protein in papers and reviews on PrP^{c}. Fortunately, a clue to the elusive prion function may lie right under, or in, our noses. LePinchon and colleagues[4] have begun this investigation in this issue

There are several major hurdles to learning about the function of a particular protein. One of these is knowing where the protein resides in cells. This localization can help narrow down the potential functions of the protein. Earlier this year, it was[5] demonstrated, using new highly specific antibodies, that PrP^{c} in the olfactory system is localized to the axons of both peripheral olfactory sensory receptor neurons and central neurons such as the mitral cells of the olfactory bulb (**Fig. 2**). Glia or support cells in the olfactory bulb or olfactory epithelium were not detectably labeled. In addition to axons, PrP^{c} was also observed in the dendritic spines of axonless olfactory bulb granule cells. These spines are both pre- and postsynaptic to mitral cells, forming reciprocal synapses. Combined with the axon staining, this suggests a potential role for PrP^{c} in presynaptic function[5]. However, given how widely expressed PrP^{c} is throughout the brain, simply showing its presence in the olfactory system was only circumstantial; further tests were required to determine whether it has a functional role in olfaction.

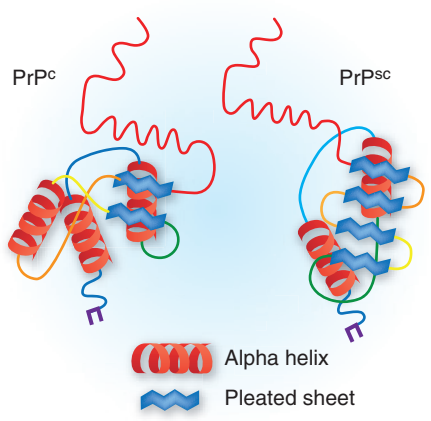

Figure 1 Representation of the cellular (PrP^{c}) and pathological (PrP^{Sc}) isoforms of prion proteins. The pleated sheets in the pathogenic form promote proteolytic-resistant accumulation in cells and extracellular plaques, triggering neurodegeneration.

The observation that PrP^{c} is expressed in olfactory sensory neurons, mitral cells and granule cells raises the possibility that it is important for the local circuit function of the olfactory bulb. Olfactory sensory neurons in the nose send axons directly into the brain, terminating on mitral cells, which send their axons directly to olfactory cortex. In the olfactory bulb, local circuits, which include granule cells, refine spatiotemporal patterns of sensory neuron input, and this local circuit function can be monitored electrophysiologically through oscillations in local field potentials.

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