# Chapter 31
# Understanding Cone Photoreceptor Cell Death in Achromatopsia

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**Abstract** Colour vision is only achieved in the presence of healthy and functional cone photoreceptors found in the retina. It is an essential component of human vision and usually the first complaint patients undergoing vision degeneration have is the loss of daylight colour vision. Therefore, an understanding of the biology and basic mechanisms behind cone death under the degenerative state of retinal dystrophies and how the activation of the apoptotic pathway is triggered will provide valuable knowledge. It will also have broader applications for a spectrum of visual disorders and will be critical for future advances in translational research.

**Keywords** Achromatopsia · Cone dystrophies · Cone photoreceptors · Cell death · Cone degeneration · Apoptosis

## 31.1 Introduction

Amongst the different neuronal cell types in the retina, photoreceptor cells are critically important as they are responsible for light detection. They form two classes, the rods and cones, with the cone cells responsible for daylight colour vision, photopic light detection and high visual acuity. In patients undergoing retinal degeneration, loss of acuity and colour vision is usually their main complaint and in some cases, vision deterioration is only reported once the degeneration has actually spread to the cones, even though the peripheral rods have been non-functional for months or even years. It is clear therefore that the quality of life of patients diagnosed with inherited retinal dystrophies would have a huge improvement if we were able to somehow preserve, or at least slow down, cone photoreceptors degeneration. However trying to understand the mechanisms behind cone cell death has turned out to be a complex web of up- and down-regulation of different cellular pathways. Several research

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groups have now made a considerable effort towards identifying and elucidating these pathways and their role in triggering cone death using different models of retinal degeneration. The aim of this review is to offer a succinct overview of some of these efforts using cone-specific degeneration models.

## 31.2 Primary Cone Loss in Achromatopsia

Complete achromatopsia (ACHM) is an autossomal recessive congenital disorder where only the cone photoreceptors are non-functional and/or undergo degeneration, while scotopic rod-mediated vision usually remains unaffected. It is mostly caused by mutations in cone-specific phototransduction genes, can affect 1:30,000 people in the US and has debilitating symptoms like severe photophobia, reduced or complete loss of colour discrimination, pedular nystagmus and severely reduced visual acuity (Michaelides et al. 2004). So far mutations in four genes have been reported to cause ACHM: cone-specific alpha transducin (*GNAT2*), the alpha and beta subunit of the cone-specific cyclic nucleotide-gated (CNG) channel (*CNGA3* and *CNGB3*) and the cone-specific phosphodiesterase alpha' subunit (*PDE6C*—reviewed in (Berger et al. 2010). All these are key players in the phototransduction cascade and essential for cone function.

The historical classification of ACHM as a stationary disorder has been due to the fact that patients usually present with either absent cone function from birth or it remaines stationary with age (Sundaram et al. 2014). This led to the belief that the cone photoreceptors in these patients did not undergo active degeneration throughout their lifetime. However, recent studies using high resolution optical coherence tomography (OCT) and adaptive optics (AO) to look at the progression of degeneration in ACHM patients showed that they can present mild to moderate morphological changes in the inner/outer segment region, substantial loss of foveal and macular cones, and, in extreme cases, hypoplasia of the retinal pigment epithelium (Thiadens et al. 2010; Genead et al. 2011; Scoles et al. 2014). Despite the controversy surrounding cone fate in human patients, in the last few years several ACHM animal models have been described and shown to have active cone degeneration: the *Cnga3* naturally-occurring mutant (Pang et al. 2010) and knockout mouse models (*Cnga3*$^{-/-}$) (Biel et al. 1999), the *Pde6c*-deficient *cpfl1* mouse and zebrafish models (Stearns et al. 2007; Chang et al. 2009) and the dog and mouse model of *Cngb3* deficiency (Sidjanin et al. 2002; Ding et al. 2009). Even though the progressive loss of cone photoreceptors was established in several of these models (Michalakis et al. 2005; Ding et al. 2009; Fischer et al. 2010; Trifunovic et al. 2010; Xu et al. 2011), the precise kinetics of the degeneration has not yet been fully elucidated.

## 31.3 PDE6 Deficiency and Cone Cell Death Mechanisms

Comparative analysis of three ACHM mouse models shows that they have a similar progression of cone death, with a sharp peak at roughly the same time around postnatal day 24 (P24) (Michalakis et al. 2005; Ding et al. 2009; Trifunovic et al. 2010), but a continual degeneration was also reported in both the *cpfl1* (Fischer et al. 2010) and *Cnga3*$^{-/-}$ models (Michalakis et al. 2005). The *cpfl1* mouse has presented itself as an ideal model for understanding the mechanisms behind cone cell death since a fast degeneration rate is coupled with the existence of the analogous, and extensively studied, rod-specific *Pde6b*-deficient *rd1* mouse.

Seminal work in this field has been published by the Paquet-Durand group at the University of Tübingen where initial studies on the *cpfl1* mouse indicated that the classical caspase-3-dependent apoptotic pathway is not activated in the degenerating cones (Trifunovic et al. 2010), mimicking previous results found in the *rd1* mouse (Paquet-Durand et al. 2009; Sancho-Pelluz et al. 2010). Not surprisingly, this suggests that the lack of a functional phosphodiesterase (PDE) might trigger similar cell death mechanism in both rods and cones. Indeed Trifunovic and colleagues were able to demonstrate that cyclic guanosine monophosphate (cGMP) accumulation, excessive activation of calcium-dependent calpains and cGMP-dependent protein kinase G (PKG) seen in the *rd1* retina (Paquet-Durand et al. 2006, 2009) was also observed in the degenerating *cpfl1* cones. They suggest that cone loss might be mediated by the phosphorylation of vasodilator-stimulated protein (VASP), a PKG substrate that has been linked to cell death: accumulation of cGMP leads to excessive activation of PKG which in turn phosphorylates VASP. However the role of intracellular calcium ($[Ca^{2+}]_i$) in the cell death mechanisms of *Pde6*-deficient photoreceptors remains unclear. A recent study using the transgenic *Pde6c*-deficient zebrafish (*Pde6c*$^{w59}$) and *rd1* mouse showed that $[Ca^{2+}]_i$ levels in mutant *Pde6c*$^{w59}$cones and *rd1* rods was not increased compared to wild-type (Ma et al. 2013a). These results challenge the prevailing view that photoreceptor degeneration due to *Pde6* mutation is driven by a global increase in $[Ca^{2+}]_i$ levels although there is strong evidence that ablating $Ca^{2+}$influx through knockout of the CNG ion channel leads to preservation of rods in the *rd1* mouse (Paquet-Durand et al. 2011). Furthermore, studies have shown that the degeneration process in *rd1* rods involves a much more complex network of interlinked players including histone deacetylases and poly-ADP-ribose-polymerase (Paquet-Durand et al. 2007; Sancho-Pelluz et al. 2010) which have not yet been investigated in the *cpfl1* retina.

An alternative mechanism for cone cell death in the *cpfl1* mouse was proposed by (Schaeferhoff et al. 2010) after showing upregulation of gene expression in cone and Müller glia cells of signal transducer and activator of transcription 3 (*Stat3*) and different components of its signaling cascade like *Cebpd*, *Socs3*, *Cntf* and *Lif*. They suggest that activation of STAT3 signaling pathways is achieved via a 28-fold upregulation of endothelin 2 (*Edn2*) which is secreted in response to photoreceptor stress. Once again there are parallels between these findings and studies in the *rd1* mouse which have shown retinal upregulation of STAT3 (Samardzija et al. 2006) and *Edn2* (Bramall et al. 2013). This suggests that *Stat3* signaling and *Edn2*

activation act as a potent cell survival response but do not however explain by which step of the active degeneration process they are triggered by. They also fail to provide evidence that *Edn2* is actually expressed in photoreceptors cells, as opposed to activated Muller glia cells found in the outer nuclear layer (ONL).

## 31.4 The Role of CNG Channels in Cone Cell Death

The extremely small number of cone photoreceptors (around 2–3 %) and lack of a macula/foveal region in the mouse retina has been a challenging and restrictive step towards studying the cone system independently. An interesting approach to overcome this has been developed by the Ding group at the University of Oklahoma where they have generated double knockout mouse lines of the *Cngb3*$^{-/-}$ and *Cnga3*$^{-/-}$ on the cone dominant *Nrl*$^{-/-}$ background (Thapa et al. 2012). These double knockout mice show equivalent impaired cone function and degeneration to their respective single CNG subunit knockouts: reduced or absent eletroretinogram (ERG) responses, reduced expression of phototransduction proteins and increased TUNEL-positive apoptotic cells in the ONL. They have used these models to show a positive correlation between cone photoreceptors CNG channel deficiency and endoplasmic reticulum (ER) stress-associated apoptosis (Thapa et al. 2012). Both models show a significant increase in ER-stress marker proteins like Grp78/Bip, CHOP, phosphor-eIF2α and phosphor-$IP_3R$; calpain II and enhanced processing of its substrate caspase-12, and the ER-stress suppressors Bcl-2 and Bcl-x proteins. The increased activation of ER stress canonical pathways in CNG deficient retinas was also shown to occur on a gene expression level (Ma et al. 2013b) and in *in vitro* testing of mutated CNGA3 subunits (Duricka et al. 2012). Interestingly, they also report nuclear translocation of mitochondria-related proteins like apoptosis-inducing factor (AIF) and endonuclease G (Endo G) (Thapa et al. 2012). This is suggestive that mitochondrial insult might have a role in the ER stress-mediated cell death process. However the fact that the levels of cytochrome *c*, caspase-3 and caspase-9 are not altered indicates that mitochondria-mediated caspase-dependent apoptotic pathways are not active in these degenerating cones. Instead, based on their results, they suggest that the ER stress observed in these degenerating cones will activate the apoptotic response by at least three separate pathways mediated by CHOP, caspase-12 and AIF/Endo G, respectively. What still remains unclear is how the ER stress is triggered in the first place. While Thapa et al. (2012) suggest three options, impaired $Ca^{2+}$homeostasis, opsin mis-localization and cGMP accumulation, their direct causality in this complex network of events requires further investigation.

Recently the role of cGMP cytotoxicity in CNG deficiency-related cell death has taken a step further as one of its major contributors (Xu et al. 2013). This recent study shows that increased levels of cGMP in *Cnga3*$^{-/-}$/*Nrl*$^{-/-}$ retinas strongly correlate with increased PKG activity and expression and coincided with apoptotic cone cell death. Further support for cGMP involvement comes from improved cone survival seen in the double *Cnga3*$^{-/-}$/*Gucy2e*$^{-/-}$ knockout mouse. *Gucy2e* encodes

retinal guanylate cyclase 1 (retGC1) and is responsible for cGMP production in cones, therefore knocking it out in *Cnga3*$^{-/-}$ retinas should lower cGMP levels counterbalance its cytotoxic effects and promoting cone survival.

## 31.5 Concluding Remarks

Recent years have seen an incredible amount of data emerge from several different studies trying to elucidate who are the key players behind cone degeneration. The studies outlined above have used a variety of different approaches both technologically and in their choice of biological system. It is reassuring however that common pathways have been reported in different models. The increased cGMP and PKG activity seen in both *Pde6c*$^{-/-}$ and *Cnga3*$^{-/-}$ retinas suggests a number of shared factors that could be involved in activating cone cell death response and therefore offers the promise of therapeutic interventions independent of the genetic lesion causing the degeneration. The role of cGMP cytotoxicity in photoreceptor cell death has already been recognized in other models of retinal degeneration like the *rd1* and GCAP1 mutants where it is clearly linked to a rise in intracellular $Ca^{2+}$ (Paquet-Durand et al. 2009). It is interesting to note however that the increased cGMP levels observed in the *Pde6c*$^{-/-}$ and *Cnga3*$^{-/-}$ models are explained by a high and low level of intracellular $Ca^{2+}$, respectively. These differences between models are supported by the fact that the separate knockout of each of the CNG channel subunits generates around 70 % of unshared genes being differentially expressed between the two models (Ma et al. 2013b). Therefore, comparisons between PDE6C- and CNG-deficiency mediated cone cell death needs to take into consideration their different roles within the phototransduction cascade and what are the functional consequences of their demise.

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