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*Review*

# Lens fibre cell differentiation and organelle loss: many paths lead to clarity

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The programmed removal of organelles from differentiating lens fibre cells contributes towards lens transparency through formation of an organelle-free zone (OFZ). Disruptions in OFZ formation are accompanied by the persistence of organelles in lens fibre cells and can contribute towards cataract. A great deal of work has gone into elucidating the nature of the mechanisms and signalling pathways involved. It is apparent that multiple, parallel and redundant pathways are involved in this process and that these pathways form interacting networks. Furthermore, it is possible that the pathways can functionally compensate for each other, for example in mouse knockout studies. This makes sense given the importance of lens clarity in an evolutionary context. Apoptosis signalling and proteolytic pathways have been implicated in both lens fibre cell differentiation and organelle loss, including the Bcl-2 and inhibitor of apoptosis families, tumour necrosis factors, p53 and its regulators (such as Mdm2) and proteolytic enzymes, including caspases, cathepsins, calpains and the ubiquitin–proteasome pathway. Ongoing approaches being used to dissect the molecular pathways involved, such as transgenics, lens-specific gene deletion and zebrafish mutants, are discussed here. Finally, some of the remaining unresolved issues and potential areas for future studies are highlighted.

**Keywords:** lens fibre cell; differentiation; development; proteolysis; apoptosis; cataract

## 1. INTRODUCTION

The lens has a unique structure consisting of tightly packed fibre cells with a specialized organization [1,2]. The fibre cells are filled with high concentrations of crystallin proteins that ensure lens transparency [3]. In most species, the lens becomes transparent during late gestational stages (early foetal in human) when the organelles and nuclei of the primary fibres have disappeared and the lens vasculature has regressed [4–7]. This transparency must be maintained throughout life in order to prevent visual impairment caused by the development of lens opacities (cataract) [8].

The mature lens is composed of two types of cells—a monolayer of lens epithelial cells up to the lens equator, which is bathed by the aqueous and the lens fibre cells. The anterior ends of the youngest cortical lens fibre cells are close to the aqueous, while the posterior ends of the same fibres are adjacent to the vitreous.

During the early stages of development, a lens vesicle forms in most species and signals from the optic vesicle induce differentiation of posterior lens epithelial cells into primary lens fibre cells, which then fill up the lens vesicle (figure 1). These central lens fibre cells (the primary fibres) remain in the centre of the lens throughout life and are therefore some of the oldest cells in the body and along with the earliest secondary lens fibre cells represent the foetal lens nucleus. As development proceeds, secondary lens fibre cells are formed from daughter cells in the meridional zone. These cells withdraw from the cell cycle and then grow out in both the anterior and posterior directions, thereby creating shell after shell of highly elongated cells to form the juvenile and adult nucleus and the cortex. Lens fibre cells comprise 99 per cent of the cells in the lens and are not restricted to the posterior pole.

Lens fibre cell terminal differentiation is accompanied by synthesis and short-range ordered packing of crystallin proteins, which provide the transparent and refractive medium through which light passes on its way to the retina [9]. Degradation of all cytoplasmic organelles (nucleus, Golgi apparatus, endoplasmic reticulum, mitochondria, etc.) within the lens fibre cells is necessary for the establishment and maintenance of lens transparency, through the creation of what has been termed an organelle-free zone (OFZ) [10]. There is evidence that the morphology of the denucleation process in primary lens fibre cells is different from the gradual fading of nuclei in secondary fibres [4,11], although this proposal requires more detailed study.

Disruptions to the process of lens fibre cell differentiation can lead to the persistence of organelles in lens fibre cells and perturbation of OFZ formation [12]. This can, therefore, contribute to inherited cataract and/or cortical cataract in adults, in which secondary lens fibre cells are still being laid down. For example, tryptophan-deficient rats develop posterior sub-capsular

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One contribution of 10 to a Theme Issue 'The ocular lens: a classic model for development, physiology and disease'.

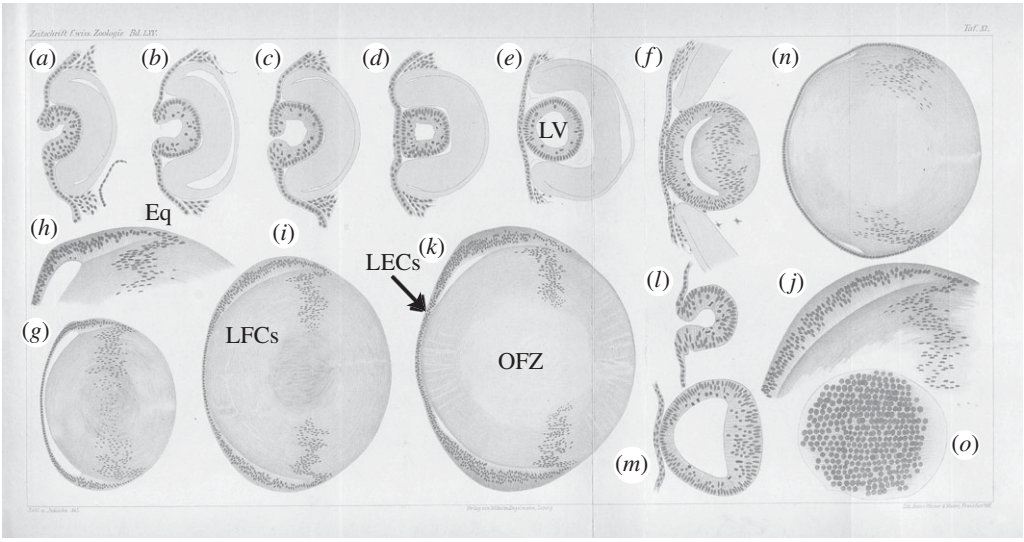


Figure 1. Carl Rabl's 1898 drawing (with additional labelling) of the developing sand lizard eye. The developing lens at different stages is illustrated in succession from left to right on both the top and bottom rows. (*a–e*) The lens invaginates from the ectoderm, then pinches off, thereby forming a hollow lens vesicle (LV). (*f*) Primary lens fibre cells (LFCs) then elongate to fill the lens vesicle. Subsequently, secondary LFCs form from lens epithelial cells (LECs) differentiating at the lens equator (Eq) or bow region (*h*). Both the primary and secondary LFC nuclei elongate and then become increasingly pyknotic and disappear, forming an organelle-free zone (OFZ), which progresses from the centre of the lens outwards as development proceeds. Image provided by Dr Ralf Dahm and used Courtesy of Zeitschrift für wissenschaftliche Zoologie.

and cortical cataracts, and this is accompanied by an enlarged lens bow with nuclear remnants, resulting from altered chromatin breakdown, which extends into the deep lens cortex [13–15]. Therefore, understanding the molecular mechanisms involved in lens fibre cell differentiation is important as it has implications for gaining further insights into certain types of cataract development as well as being a relatively simple system amenable to molecular and developmental analysis.

The loss of organelles, with the maintenance of cell structure, only occurs in a few other cell types, e.g. erythrocytes and keratinocytes [16]. Interestingly, a similar process also occurs in the development of plant phloem sieve elements [17]. The central vacuole breaks down so that cytoplasmic ribosomes, Golgi bodies and the nucleus are degraded and eventually eliminated. Filamentous phloem proteins (P-proteins), however, survive the autophagic process and the endoplasmic reticulum is retained in the mature sieve elements, although in a highly modified form.

The process of lens fibre cell differentiation accompanied by organelle degradation has been reviewed on a number of previous occasions [8,9,11,18–23]. Here, an overview of this phenomenon is provided along with recent insights into the biochemistry and molecular biology of this process as well as consideration of the networks of signalling molecules involved, remaining unresolved issues and thoughts on future studies.

## 2. HISTORICAL OVERVIEW OF MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF LENS FIBRE CELL ORGANELLE LOSS

It was first noted by Meyer in 1851 that the central cells of the dog lens are devoid of nuclei, but the first studies to definitively and systematically examine organelle degradation in the developing lens were those of Carl Rabl between 1897 and 1899 (reviewed in [24,25] and figure 1). Rabl examined numerous species and showed that similar patterns of organelle loss were occurring in virtually all of them; however, moles, which are virtually blind, were an exception.

In their famous lens-reversal transplant experiment, in which the lens of the 5 day chick embryo was surgically reversed so that its epithelium faced the neural retina, the Coulombres showed that the elongation of those lens cells (which had already differentiated) was arrested and the epithelial cells differentiated into a new set of lens fibre cells [26]. They clearly showed that by 10 days post-operation, the newly differentiated lens fibre cells (derived from the lens epithelium closest to the retina following the transplantation) were undergoing the process of denucleation, as indicated by pyknotic nuclei, particularly at their centres. Thus, all the lens epithelial cells are competent to undergo differentiation into lens fibre cells (including nuclear degradation) in response to diffusible factors produced by the retina acting on the posterior of the developing lens.

However, the first studies to carry out a thorough spatio-temporal analysis of the breakdown of the lens fibre cell nuclei in detail were those of Sohan Modak *et al.* in the late 1960s–1970s using the chick embryo as a model [27]. This group also demonstrated that fragmentation of DNA occurs during lens fibre cell degeneration [28] and that this fragmentation is associated with single-strand breaks and the release of free 3′-OH ends, which then can act as templates for calf thymus DNA polymerase and terminal deoxynucleotidyl transferase [29,30] in an early version of the TUNEL reaction [31]. Subsequently, double-strand DNA breaks occur resulting in the presence of low-molecular-weight DNA species of discrete sizes [32], and this is similar to the well-characterized banding patterns seen in DNA from cells undergoing classical apoptosis.

The breakdown of nuclei in mouse secondary lens fibre cells was examined [33]. It was revealed that fragments of the nucleolus remain in the denucleated lens fibre cells even when differentiation is complete and, further, it was suggested that disturbances in denucleation might contribute towards cataractous changes in the lens. Indeed, subsequently, the same group and others revealed that accumulation of nuclear and mitochondrial fragments occurs in congenital and cortical cataract owing to incomplete organelle degradation in the equatorial region of the lens [34–36]. Moreover, maternal rubella virus infection causes bilateral congenital cataract [37], including defects in lens fibre cell organelle degradation; in fact, the retention of nuclei in surviving lens fibre cells is characteristic of rubella infection [38].

Changes in lens fibre cell nuclear morphology could also be observed histologically after three to four weeks in explanted embryonic chick lens epithelia *in vitro* [39] and in rat lens epithelial explants [40]. Degeneration of nuclei also occurs in chick lens epithelial cells differentiating *in vitro* into structures called lentoids composed of lens fibre-like cells [41,42]. Following cataract surgery, lens epithelial cells remaining in the capsular bag can undergo transdifferentiation accompanied by organelle degradation, and, in this way, form a peripheral ring of lens-fibre-like cells called Soemmering's rings [43].

## 3. UNRESOLVED ISSUES

A major remaining issue is whether the loss of lens fibre cell organelles can truly be thought of as a form of so-called 'attenuated apoptosis' [21]. This term was suggested since, unlike in 'classical apoptosis', the lens fibre cells from which the organelles have been removed remain following activation of apoptosis signalling pathways, rather than undergoing apoptosis followed by engulfment and digestion by invading macrophages. Morphologically, the degenerating nuclei in lens fibre cells resemble those undergoing apoptosis, as chromatin condensation and marginalization occur as well as DNA fragmentation between nucleosomes, thereby producing the characteristic laddering pattern of apoptosis seen in agarose gel electrophoresis [10,44].

However, there are clear differences between classical apoptosis and lens fibre cell organelle loss. The process of denucleation and organelle loss takes place over several days, rather then several hours, as is the case with apoptosis [8]. In classical apoptosis, the cytoskeleton of the dying cell is completely degraded (this is part of the cellular dismantling process), whereas in lens fibre cells the cytoskeleton is maintained, even though organelles have fully disappeared [21,45]. Moreover, phosphatidylserine flipping to the outer leaflet of the membrane of the lens fibre cells does not occur as it normally does during apoptosis [10,42]. These considerations all suggest an underlying difference in the way these pathways are controlled in differentiating lens fibre cells versus cells undergoing classical apoptosis. However, given the proficiency with which cells can use the same proteins and pathways in widely different contexts, it is likely that there is substantial overlap and redundancy in the signalling pathways employed in both processes (denucleation/organelle loss and apoptosis). A case in point is that of the caspases (cysteine-dependent aspartate-directed proteases), with essential roles in apoptosis, necrosis and inflammation.

Caspases are generally considered to be instrumental in bringing about the cascade of proteolytic activities that ultimately result in the morphological characteristics of apoptosis [46]. Despite earlier reports implicating caspases in lens fibre cell organelle loss (which will be discussed in more detail below), it has been demonstrated that knockout mice lacking in caspase-3 and caspase-6 do not have defects in lens fibre cell organelle loss, and thus have normal lenses [47]. However, owing to the difficulty in raising large numbers of animals simultaneously deficient in both genes, the authors were unable to test whether there was a compensatory increase in the activity of other caspases (or indeed other proteases, such as calpains for example).

Moreover, there is emerging evidence that controlled apoptosis signalling actually has a more general role in lens fibre differentiation. Therefore, one of the ongoing problems in identifying factors that are directly involved in organelle loss is that knockout of genes that are primarily involved in promoting lens fibre cell differentiation will also result in maintenance of organelles in lens fibre cells, as a secondary consequence of their absence.

In an evolutionary context, it is highly likely that there is great redundancy and pathway parallelism built into this developmental system, such that the deactivation of one pathway leads to the activation of alternative (e.g. caspase-independent) mechanisms, which are able to facilitate lens fibre cell differentiation and/or organelle loss in the absence of the inactivated gene(s). Furthermore, given the number of different caspases and their functional redundancy, it is possible that even multiple caspase knockouts would not be capable of accounting for all the players involved. Indeed, cross-breeding constraints make it very difficult to derive more than triple knockouts in a financially feasible manner, meaning that the roles of individual components of very redundant systems may be very hard to tease out using genetic mutants, despite their definite value.

Indeed, there is emerging support for the argument that multiple and redundant networks of interacting proteins/signalling molecules are involved in certain important aspects of development [48]. These authors point out that, up until now, signal transduction has largely been regarded as a linear process, but that more recent data from large-scale and high-throughput experiments indicate that there is extensive cross-talk between individual signalling cascades, leading to the notion of signalling networks. Two cases in point are Wnt signalling, in which the subdivision into canonical and non-canonical Wnt signalling pathways solely based on the identity of Wnt ligands or frizzled receptors is not appropriate anymore (these pathways are redundant and overlap), and also the network of transcription factors involved in early eye development and disease [49–51]. Importantly, in the context of lens

fibre cell differentiation and organelle loss, it is becoming apparent, using systems biology approaches, that apoptosis signalling and the proteasomal system can also be considered to be gene networks [52,53]. It is likely that such signalling networks are very robust when it comes to coping with mutations in, or deletions of, important genes within them that contribute towards a specific developmental process, such as lens development.

Other challenges include not only the identification of the factors involved and their place in the signalling networks, but also the identification of how these factors are activated, integrated and coordinated spatially within the lens fibre cells during their differentiation. This includes their associations with subcellular organelles, thereby determining the means by which the lens fibre cells undergo organelle loss, rather than complete 'classical' apoptosis. Indeed, the data suggest that, under normal circumstances, lens fibre cells are supremely resistant to classical apoptosis, although they may be pushed into a classically apoptotic programme under certain circumstances, e.g. when undergoing cellular stress, such as an unfolded protein response, owing to the over-expression of certain transgenes expressing wild-type proteins [54].

In addition it is likely that there are cross-species differences that also complicate the picture; for example, the morphology of nuclear degradation in chick lens fibre cells is clearly different from that in mouse lens fibre cells (compare [4] and [42]). Finally, it needs to be taken into account that the development of the zebrafish lens occurs differently than in most other vertebrates in that a lens vesicle does not form, rather the lens is derived from the sorting out of cells within a ball that pinches off directly from the ectoderm [7,55].

## 4. OVERVIEW OF FACTORS INVOLVED IN LENS FIBRE CELL DIFFERENTIATION AND TRIGGERING AND COORDINATION OF ORGANELLE LOSS

Although the process of organelle degradation in lens fibre cells does not appear to be a direct form of conventional apoptosis that definitively uses caspases, numerous apoptosis-related genes are expressed in the lens [6,56–58]. Further investigation is required to clarify the role of apoptosis signalling components in the lens and their potential roles in the removal of lens fibre cell nuclei and organelles and/or lens fibre cell differentiation in general.

### (a) *The B-cell lymphoma 2 family*

B-cell lymphoma 2 (Bcl-2) is the founding member of the Bcl-2 family of apoptosis regulator proteins. Bcl-2 family members have roles in the mitochondrial cell death pathway. We and others have demonstrated that Bcl-2 members are expressed in a spatiotemporally regulated manner during chick lens development [6,58]. Pro-apoptotic members of the family were upregulated in the lens and anti-apoptotic members were downregulated at the time that organelle loss commences in the chick embryo at embryonic day 12 [6].

Further work *in vitro* and *in ovo* revealed that over-expression of Bcl-2 in the chick lens results in morphological defects, including a more spherical shape, disorganized lens fibre cells and disruption of lens fibre cell denucleation, as revealed in the lens core by a failure of the nuclear chromatin to condense [59]. Over-expression of Bcl-2 reduced caspase-9 activation and cleavage of the caspase substrate DFF45. A role for Bcl-2 family members in lens fibre cell differentiation was also demonstrated in mice [60]. In this study, mice were generated exhibiting lens-specific Bcl-2 expression, and this interfered with normal lens fibre cell differentiation, inducing cataracts, microphakia, vacuolization, lens fibre cell disorganization and inhibition of lens fibre cell denucleation.

Weber & Menko [58] extended these studies in an elegant series of experiments, identifying a novel role for the mitochondrial cell death pathway in the initiation of the signalling of lens fibre cell differentiation. These authors showed that there was an increase in expression of pro-apoptotic Bcl-2 family members (Bax and Bcl-Xs) at the equatorial region where the lens fibre cells were beginning to differentiate, suggesting that these pro-apoptotic molecules provide the initial signal for lens fibre cell differentiation. Cytochrome c was released from mitochondria in the equatorial region and the cortical fibre zone and mitochondrial membrane potential was progressively diminished as cells in the equatorial region differentiated into cortical lens fibre cells, until the mitochondrial membrane potential of cells in the nuclear region had completely disappeared. In addition, anti-apoptotic members of the Bcl-2 family (Bcl-2 and Bcl-$x_l$) were also expressed during the early stages of lens fibre differentiation. They were released from the pro-apoptotic protein Bad as this process was initiated, facilitating the controlled release of cytochrome c from mitochondria, thereby potentially allowing Bax to activate lens fibre cell differentiation without actually tipping the balance all the way to apoptosis. These authors also used the apoptogen staurosporine at low concentrations in short-term exposure experiments on chick lens epithelial cell cultures. Such exposure activated cell death signalling pathways through the mitochondria and upregulation of markers of lens fibre cell differentiation occurred, including filensin, CP49, aquaporin 0 and delta-crystallin.

### (b) *Tumour necrosis factors and related molecules*

Tumour necrosis factors (TNFs) belong to a family of cytokines implicated in inflammation and cell death. TNF-related factors are expressed in the lens during development [42]. The extent of nuclear degradation in differentiating chick lens epithelial cell cultures could be influenced by the addition of TNFα and antibodies to TNFα as well as agonistic and antagonistic antibodies to TNF receptors. We have recently further examined the expression of a number of members of the TNF family and associated signalling molecules in the chick embryo lens at various stages using RT–PCR ([61], figure 2 and table 1). These studies demonstrated that TNF-related molecules and their signalling components are expressed in the developing

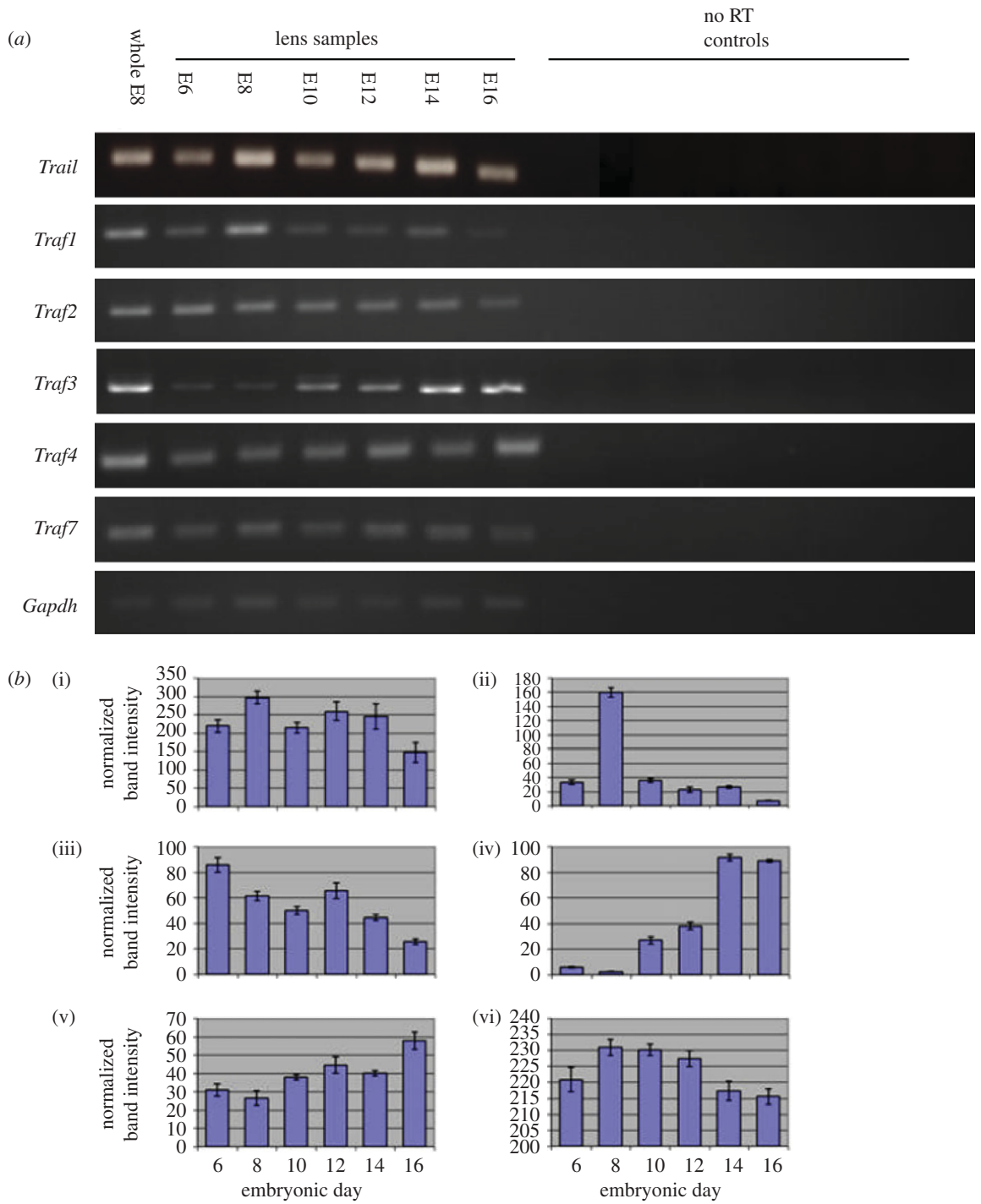


Figure 2. Semi-quantitative RT–PCRs with no RT controls for TNF-related genes expressed during chick embryo lens development. (*a*) PCRs presented are representative of three replicates. (*b*) Graphical representation of relative mean expression levels normalized with respect to *Gapdh* in each case, using densitometry. PCR reactions carried out in triplicate. Error bars represent standard error of the mean. See table 1 for details of primers, annealing temperatures and number of cycles used for each primer. RNA was isolated from pooled lenses using TRIzol (Invitrogen, Paisley, UK); reverse transcription (RT) reactions were carried out using the Superscript II Reverse Transcriptase kit (Invitrogen); PCR was carried out using GoTaq Flexi DNA Polymerase (Promega, UK). PCRs were visualized using 2% agarose gel electrophoresis; densitometry was carried out using Scion Image software [61]. (i) *Trail*; (ii) *Traf1*; (iii) *Traf2*; (iv) *Traf3*; (v) *Traf4*; (vi) *Traf7*.

lens and therefore support the suggestion that TNF-related signalling is potentially involved in regulating organelle loss in lens fibre cells and/or lens fibre cell differentiation. The results therefore suggest that organelle loss could be a process activated by TNFα-mediated cell signalling cascades, although the involvement of a passive process (such as a reduction in oxygen tension at a specific point within the lens fibre cell mass) cannot be ruled out [62].

Further recent evidence has emerged to support a possible role for TNFα signalling in lens fibre cell differentiation and/or organelle loss. Persistent FoxE3 expression blocks organelle degradation during lens fibre cell differentiation, as indicated by the lack of

Table 1. List of TNF-related primer gene sequences, band size, optimized annealing temperature and cycle number for PCRs presented in figure 2. Primers were designed using Primer3 [61].

| gene | accession number | band size | cycles | annealing temperature | forward primers | reverse primers |
|---|---|---|---|---|---|---|
| *Gapdh* | NM_204305.1 | 169 | 18 | 61 | GGAGAAACCAGCCAAGTATGATG | AAAGGTGGAAGAATGGCTGTCA |
| *Traf1* | XM 415406 | 183 | 30 | 60 | GAATGGAGATGGGATGGGAAA | TGAAAGGAAGCAGAAGCCAAG |
| *Traf2* | XM 415561 | 260 | 32 | 62 | CTTCCTGCCATTCCCTCATC | GCACCACACACCTTGTTACTC |
| *Traf3* | XM 421378 | 658 | 28 | 62 | TCAGGGAACAAACCAACAAA | AAGGTAAACTCTGGCACACATC |
| *Traf4* | XM 415823 | 204 | 27 | 62 | AGGAGAGCACCAAGGCACAC | TGTAGAAAGGAGGGCTGAAGAA |
| *Traf7* | NM 001012528.1 | 228 | 28 | 62 | AACGGGACAAGAATGGAAACA | GCAATGAACGGACGGAGATAG |
| *Trail* | NM 204379 | 146 | 26 | 62 | GGAACGAATAAAGAATCCCAAG | CGCCCTGGTAGACGGAATAAA |

an OFZ in mice expressing *Foxe3* in lens fibre cells from which it is normally lost during development [63]. This was due, at least in part, to shifts in the interconnected pathways of TNFα and apoptosis signalling, away from pro-apoptotic programmes towards anti-apoptotic programmes, indicating an involvement for pro-apoptosis signalling (possibly through TNF-related pathways) in lens fibre cell differentiation.

Support for a general role for TNF-related signalling in lens fibre cell differentiation comes from a recent study investigating cytoplasmic activation/proliferation-associated protein-2 (Caprin2), which contains domains belonging to both the Caprin family and the C1q and TNF super-family [64]. Caprin2 is expressed in the differentiating lens fibre cells and this expression is fibroblast growth factor (FGF)8 dependent. Caprin2 is subsequently downregulated, and this is spatially and temporally coordinated with the onset of lens fibre cell denucleation and terminal differentiation. Thus, Caprin2 may play a role in the differentiation of lens fibre cells prior to denucleation.

This putative role of TNF and/or pro-apoptosis signalling pathways as a general requirement(s) for lens fibre cell differentiation is interesting in the light of accumulating evidence indicating that regulated, low-level apoptosis signalling influences cellular differentiation [58]. These authors have termed this apoptosis-related Bcl-2- and aaspase-dependent (ABC) differentiation. This occurs in a number of different cell types, for example during differentiation of keratinocytes, monocyte differentiation into macrophages and during erythroid cell differentiation.

### (c) *The p53 family and regulators*

p53 is a tumour suppressor protein that is important in regulating the cell cycle and is also involved in DNA repair and apoptosis. It thus functions as a tumour suppressor with a role in preventing cancer. The developmental roles of p53 have not been extensively studied. Homozygous p53-deficient mice were shown to be susceptible to spontaneous tumours, but were otherwise viable and appeared to develop normally [65]. However, subsequently, it was demonstrated that a subset of p53-deficient embryos exhibited exencephaly and died *in utero* [66]. Furthermore, severe ocular defects including lens capsule disruption and posterior migration of the lens epithelium (indicative of cataract formation) were observed in p53-deficient mice on a C57BL/6J (B6) background, but not on the 129/SvJ background [67]. It is therefore possible that there may be hitherto undetected subtle developmental alterations in lens OFZ formation in certain strains of p53-deficient mice, but this has not been investigated.

We carried out array analysis to identify numerous apoptosis genes expressed in the mouse lens during development and early postnatal maturation and further analysed spatio-temporal regulated patterns of expression of p53 and Mdm2 (a ubiquitin ligase that regulates the activity and amount of p53) [57]. Our studies built on previous work showing that p53

is expressed in the lens epithelial cells of the central and pre-equatorial zones and in the lens fibre nuclear bow region, and that temporally distinct patterns of p53-dependent apoptosis have been identified during mouse lens development [68–70].

Over-expression of human wild-type p53 in the mouse lens results in microphthalmia owing to apoptosis of lens fibre cells after birth instead of differentiation [71]. Intriguingly, in this study, over-expression of a human mutant p53, which inhibited endogenous p53 function, resulted in the presence of nuclear cataracts in these mice owing to the persistence of remnants of nuclei, thereby directly implicating p53 signalling in lens fibre cell nuclear degradation.

We are currently using the Mdm2 inhibitor nutlin-3 on chick embryo lens organ cultures and have generated preliminary data indicating that inhibition of Mdm2 results in expansion of the OFZ in these lenses, possibly through a p53-dependent pathway (A. McEnroe & M. A. Wride, unpublished data). Indeed, Mdm2 and p53-dependent apoptosis signalling appear to play a role in erythroid differentiation, which is also accompanied by denucleation [72,73].

Finally, it has been proposed that phosphatidylinositol 3-kinase (PI3K) may regulate glycogen synthase kinase (GSK) signalling during lens fibre cell differentiation, and this could, in turn, influence p53 signalling through multiple mechanisms including preventing p53 degradation, activating p53 phosphorylation and changing p53 distribution [74]. Thus, the regulation of GSK via the PI3K pathway could be important in controlling the amount of p53 and its localization and function during lens fibre cell differentiation. It was proposed by these authors that, whereas GSK3 may promote p53 activity to promote lens fibre cell differentiation, the PI3K survival pathway could regulate GSK3 to prevent commitment to classical apoptosis. These suggestions merit further attention in view of the potential emerging roles for p53 in lens development discussed here.

### (d) *Globins as apoptosis signalling regulators in the lens?*

During microarray studies investigating gene expression changes during the development and maturation of the mouse lens and during cataract progression in the Sparc knockout mouse, we identified differential expression of various haemoglobin isotypes in the lens [56,75]. Subsequently, we revealed spatio-temporal patterns of both foetal- and adult-haemoglobin subunit expression in the mouse lens during development, postnatal maturation and in the adult [76]. Haemoglobin subunit proteins were expressed in lens epithelial cells (cytoplasmic) and in cortical lens fibre cells (perinuclear and cell-surface-associated). The pattern of expression of haemoglobin subunits in the perinuclear region in cortical lens fibre cells was correlated with denucleation and could indicate an involvement in this process, since, in the absence of haem, α-globin promotes apoptosis signalling [77,78]. Indeed, in our studies, a sensitive haem assay demonstrated negligible levels of haem in the developing lens postnatally. The results suggest that haemoglobin protein subunits may not be associated with haem and have novel developmental roles outside their well-known roles in the haematopoietic system. Given the absence of the haem group, at least some of these functions may be independent of oxygen metabolism and may include regulation of apoptosis signalling during lens fibre cell differentiation. Finally, there is evidence of cataract associated with haemoglobinopathies such as sickle cell anaemia and both α- and β-thalassaemias (see [76] for further discussion).

### (e) *Autophagy*

A potential role for autophagy in lens organelle degradation has been investigated [79]. Autophagy is a cellular bulk degradation system through which cytoplasmic components are degraded within lysosomes, so it was thought that this might be an ideal mechanism for lens fibre cell organelle degradation. However, although autophagy occurs in embryonic lens cells, organelle loss occurred normally in autophagy-deficient Atg5−/− mice (Atg5 is a protein known to be essential for autophagosome formation). Given the potential redundancy in the various pathways involved in organelle loss, the potential role of other degradative and proteolytic pathways has also been examined.

## 5. PROTEOLYSIS, DNA DEGRADATION AND DNA REPAIR

### (a) *Caspases and the apoptosome*

Both upstream initiator and downstream effector caspases are expressed in the lens [6,47,80,81] and caspase substrates are cleaved during the formation of the OFZ: poly (ADP-ribose) polymerase (PARP), lamin B and DNA fragmentation factor (DFF), although such substrates may also be cleaved by other proteases such as calpains for example [6,10,47,80]. Functional evidence suggestive of a role for caspases in lens fibre cell differentiation was provided by studies in which synthetic peptide inhibitors of various caspases significantly reduced organelle loss in lens cell cultures [6,80].

#### (i) *Caspase-3, -6 and -7*

It was further suggested that caspase-3 activity may be associated with differentiation of lens epithelial cells into lens fibre cells, rather than directly with lens fibre cell organelle loss, in studies in chick examining the involvement of the intrinsic mitochondrial pathway in lens fibre cell differentiation [58]. Caspase activity appeared to be required for lens fibre cell differentiation *in vitro*. The addition of caspase inhibitors resulted in suppression of lentoid formation. However, the expression of markers of lens fibre cell differentiation (CP49, aquaporin 0 and delta crystallin) was unaffected, suggesting a potential involvement of caspases in the morphological changes associated with lens fibre cell differentiation in the chick. It was subsequently demonstrated that actin filament disassembly induces lens fibre cell differentiation, that this was coincident with the activation of caspase-3 and that the actin filaments themselves provide an essential survival signal during lens fibre cell differentiation that may be conveyed via Bcl-2

and/or inhibitors of apoptosis (IAPs) [82]. Subsequently, the same group showed that during the early stages of lens fibre cell differentiation, actin reorganization was dependent upon PI3K signalling [74]. During later stages of lens fibre cell differentiation, at the time of organelle loss, PI3K had a protective function (preventing full apoptosis) by signalling survival through activation of its downstream effector GSK3 (as discussed previously).

Members of the IAP family, including cIAP1, cIAP3 and Survivin, were also associated with the equatorial region of the lens in which lens fibre cell differentiation is beginning, and were upregulated in lens epithelial cell cultures in response to the addition of the lens fibre cell differentiation factors FGF and insulin-like growth factor (IGF) [58]. It was suggested that tight regulation of low-level caspase activity by IAPs in early differentiating lens epithelial cells at the equatorial region of the lens is necessary for differentiation into lens fibre cells, rather than apoptosis of these cells. Thus, the IAPs could be working in harmony with the Bcl-2 family members (as described above) to bring about differentiation of lens fibre cells, rather than apoptosis.

Gap junctions have a pivotal role in maintaining cell–cell communication and lens transparency [83]. A role for caspases in chick lens fibre cell differentiation is further supported by studies examining their role in connexin (Cx; the subunits of which gap junctions are composed) cleavage during lens fibre cell differentiation. Caspase-3 causes truncation of Cx45.6 during chick lens fibre differentiation [84,85]. In addition, caspases are involved in proteolysis of lens membrane components, such as spectrin [86].

A caspase reporter assay revealed an increase in caspase-6-like activity (as indicated by increased VEIDase activity) correlating with organelle loss in lens fibre cells in the presence of a proteasome inhibitor (lactacystin) [87]. In the same report, over-expression of the human papilloma virus type 16 E7 gene in the lens fibre cells resulted in significantly increased caspase-3 activity and classical apoptosis of the lens fibre cells. Thus, these studies suggested a differential role for several caspases during lens fibre cell differentiation: caspase-6 in lens fibre cell denucleation, whereas over-expression of caspase-3 caused apoptosis. However, there may be other reasons for these results, as will be discussed below.

Caspase activities in the lens are regulated by αB crystallin [88]. Classical apoptosis rather than lens fibre cell denucleation/organelle loss occurred in the lens fibre cells of αA-/αB-crystallin double-knockout mice, owing to an increase in caspase-6 activity in lens fibre cells in the absence of crystallins. αA-crystallin interacted directly with caspase-6 to suppress its enzymatic activity, thereby potentially promoting organelle loss in lens fibre cells rather than apoptosis *per se*. Interestingly, lens gene expression analysis in the blind cavefish *Astyanax mexicanus* indicated that downregulation of αA-crystallin was associated with the onset of lens apoptosis and degeneration, and that this does not occur in the surface-dwelling form of the cavefish in which the eyes do not degenerate [89]. Since αA-crystallin is a member of the small heat shock protein (HSP) family and HSPs regulate both the intrinsic and extrinsic pathways of apoptosis signalling through inhibition of proteolytic maturation and/or activity of caspases and non-caspase-dependent cell death pathways, the role of HSPs in lens fibre cell differentiation and organelle loss deserves further investigation.

However, as previously mentioned, the data relating to the potential role of caspases in denucleation/organelle loss and lens fibre cell differentiation are somewhat inconsistent. This situation is further complicated by the use of different species. The role of executioner caspases (caspase-3, -6 and -7) in lens fibre cell organelle loss has been examined using caspase knockout mice deficient in each of the executioner caspases compared with age-matched wild-type lenses [47]. No significant differences between the size and shape of the OFZ were observed between the individual knockouts or caspase-3 and caspase-6 double knockouts and the wild-type mice, indicating that executioner caspase-3 and -6 are not essential for lens organelle degradation at least in the mouse. Moreover, the cleavage patterns of both $\alpha_8$-connexin and DNA fragmentation were examined using western blotting. As expected, both proteins were truncated during fibre cell differentiation in wild-type lenses, but the cleavage pattern of these substrates in the caspase-3 knockout mice was indistinguishable from the wild-type lenses, implying that alternative proteolytic mechanisms were involved in this truncation event. The only defect observed in the caspase-3 knockout mice was anterior polar cataract, possibly resulting from lack of apoptosis in the lens epithelium in the caspase-3 knockouts. The results, therefore, implicate caspase-3 in some aspect(s) of the maintenance of lens epithelium integrity rather than as factors involved in lens fibre cell differentiation/organelle loss at least in mice.

This conclusion is supported by studies using procaspase-3 transgenic zebrafish [90] in which although alterations were observed in the morphology of numerous tissues in the embryo, the developing lens was unaffected, suggesting an intrinsic resistance of the zebrafish lens to caspase-3 activity. As well as those issues discussed previously, it is also possible that different pathways contribute to and/or compensate for the lack of certain genes (e.g. in knockout studies) to varying degrees during lens fibre cell differentiation/organelle loss in different species.

(ii) *Caspase-9 and the apoptosome*

Caspase 9 is expressed and activated before embryonic day 12 in intact chick embryo lenses, and it was proposed that it has a role in lens fibre cell organelle loss/denucleation through the intrinsic mitochondrial pathway and apoptosome [81]. Caspase-9 was localized in the cytosol outside mitochondria in non-differentiating cultured lens epithelial cells, whereas in lens fibre cells caspase-9 migrated into mitochondria after the latter underwent the membrane permeability transition that is characteristic of apoptotic cells. At the same time, caspase-9 was colocalized in the cytosol of lens fibre cells, with cytochrome c released from the mitochondria after the mitochondrial membrane permeability transition and during the period of nuclear

shrinkage. Coincidently, mitochondria were seen to aggregate around degenerating nuclei and, at the same time, cytochrome c disappeared rapidly; subsequently, mitochondrial breakdown occurred approximately coincident with nuclear degradation. Apaf-1, another cytosolic protein of the apoptotic cascade, also migrated to the permeabilized mitochondria and also colocalized with caspase-9 and cytochrome c in the cytosol or mitochondria of denucleating cells forming an 'apoptosome' in these cells, as in apoptotic cells. Interestingly, in these experiments, no migration to the nucleus of caspase-9 or Apaf-1 was observed (migration to the nucleus of caspase 9 and Apaf-1 has been demonstrated in cells undergoing classical apoptosis [91]). Therefore, the absence of translocation of caspase-9 to the nucleus, combined with the early permeability changes in the lens fibre cell mitochondria, could be responsible for the inability of the apoptotic plasma membrane events to follow nuclear degeneration in lens fibre cells, owing to failure to activate nuclear–cytoplasmic signalling pathways. This is an interesting suggestion, since the mechanisms by which the events of nuclear degradation are decoupled from the plasma membrane events in lens fibre cells remain a mystery.

Apaf-1 knockout mice exhibit lens fibre cell differentiation defects [92]. It is of relevance and interest to the above results that Apaf-1 has recently been shown to have an additional non-apoptotic function as a regulator of the DNA damage checkpoint [93]. Since Apaf-1 is apparently not found associated with the nuclei of lens fibre cells [81], it is tempting to speculate that this could lead to an inability to repair the DNA strand breaks associated with denucleation, thus leading to nuclear degradation in lens fibre cells [32,94], especially since DNA repair mechanisms are impaired during lens fibre cell differentiation [95]. Indeed, the DNA repair molecule Nbs1 has been shown to have a hitherto unexpected role in terminal differentiation of lens fibre cells and cataractogenesis [96]. All Nbs1 knockout mice develop cataracts at an early age owing to altered lens fibre cell differentiation, including disruption of normal lens epithelial and fibre cell architecture and incomplete denucleation of lens fibre cells.

(iii) *A role for caspases or not?*

Thus, overall, the role of caspases in lens fibre cell denucleation/organelle loss is still not entirely clear. The data clearly show that the effectors caspase-3, -6 and -7 do not have a role in this process in the mouse, but that caspase-3 may have a role in regulating classical apoptosis in the developing lens epithelium. However, before a role for caspases in this process can be definitely ruled out, it will be necessary to examine the lenses of caspase-1, caspase-2, caspase-4 and caspase-9 knockout mice for defects in OFZ formation (as well as multiple knockouts thereof), since each of these caspases was implicated in lens fibre cell differentiation/organelle loss in the chick embryo [6,81]. Although it is generally understood that these so-called initiator caspases feed into the effector caspases, this may not necessarily always be the case. For example, caspase-2 displays features of both the initiator and executioner caspases [97]. Moreover, it is likely that there are species-specific differences in the pathways involved; the chick studies imply a role for various caspases in lens fibre cell differentiation/organelle loss, while the mouse studies so far performed do not. However, given the involvement of networks of signalling factors, it is also possible (as previously mentioned) that the lack of caspases in the knockouts is functionally compensated for by concomitant upregulation and/or activation of other caspases or other non-caspase pathways.

In the light of this, the potential involvement of such alternative proteolytic pathways in nuclear and organelle degradation during lens fibre cell differentiation has been investigated during lens fibre cell differentiation, including cathepsins, calpains and the ubiquitin–proteasome pathway (UPP).

### (b) *Cathepsins*

Cathepsins are lysosomal proteases that are involved in the regulation of apoptosis [98]. They also have roles in developmentally programmed cell death [99,100] and in eye physiology and pathology [22,23,101].

We demonstrated that an inhibitor of cathepsin B reduced the extent of denucleation in differentiating chick lens epithelial cell culture by 40 per cent [6]. We therefore suggested in that report that cathepsins may have a role in organelle loss and, indeed, recent data support this suggestion.

The lysosomal system plays a role in the degradation of cellular organelles during lens cell differentiation. DNase II-like acid DNase (DLAD) knockout mice exhibit cataracts as a consequence of persistent DNA in all lens fibre cells [102]. The distribution and activity of DLAD in lens fibre cells has now been examined [103]. DLAD activity correlated with and was necessary for lens fibre cell denucleation and was most probably contained initially in lens fibre cell lysosomes before release into the cytoplasm. Indeed, it has been demonstrated that DLAD is synthesized as a precursor with a signal sequence, and is localized to lysosomes in differentiating lens fibre cells, but not in lens epithelial cells [104]. Moreover, DLAD colocalized with lysosomal-associated membrane protein 1 (Lamp-1) in the lysosomes of lens fibre cells in a narrow region bordering the OFZ, and was also detected in degenerating nuclei. Lysosomes appeared to fuse with the lens fibre cell nucleus prior to chromatin degradation. Microarray studies comparing gene expression profiles between lens epithelial cells and cortical lens fibre cells indicated that genes for lysosomal enzymes, including cathepsins, were strongly upregulated in lens fibre cells.

Mouse embryonic stem (ES) cells can be induced *in vitro* to form eye-like structures, including lens-like structures that are positive for structural proteins including crystallins [105,106], although the cultures were not examined for denucleation events *per se*. Recently, such studies, indicating the ability of ES cells to recapitulate lens development *in vitro*, have been extended using human ES cells [107]. Elongated fibre-like cells showed loss of cytoplasmic organelles and condensation and disintegration of nuclei. In addition, both electron-dense and fragmented nucleoli were found in individual lens fibre cells. Thus, the lens fibre cells observed in these studies could be undergoing the early stages of terminal

differentiation. Given that DLAD has been implicated in lens fibre cell DNA fragmentation [102–104], it was intriguing that, using qRT–PCR, there was only a two-fold increase in DLAD transcripts at day 35 of ES cell culture compared with earlier stages of differentiation. The authors suggested that the lack of significant upregulation of DLAD at the mRNA level might be the reason that nuclei were still retained in most of the lens fibre cells in lentoid bodies at the stages examined, although a definitive answer to this would require examination of DLAD protein levels. It is also possible that a longer time course of ES cell differentiation may be required in this system in order to see significant upregulation of DLAD and, therefore, complete lens fibre cell denucleation and/or that an additional nuclease has a role in human lens fibre cell denucleation, although this seems less likely.

### (c) *Calpains*

Calpains are a family of calcium-dependent, non-lysosomal cysteine proteases that are expressed in a wide range of tissues. There is abundant evidence that calpain-mediated proteolysis is important during cataract progression [108–113], and novel calpain inhibitors are being developed as potential new treatments for cataract [114,115]. There is also now emerging evidence of a role for calpains in lens fibre cell differentiation and/or organelle loss.

Dynamic changes have been observed in expression of various lens-specific calpains (Lp82 and Lp85) and ubiquitous calpains (calpain-2 and calpain-10) during mouse embryo lens development and postnatal mouse lens maturation [116]. In this study, calpain-2, calpain-10 and Lp85 were expressed in dynamic patterns in lens epithelial cells and peripheral lens fibre cells, indicating a potential role in lens development. Calpain-2 was also expressed in the lens epithelial cells and in the equatorial transition zone at two weeks of age. Calpain-10 and Lp85 were primarily localized to nuclei in the equatorial region of the lens. Therefore, it was suggested that these calpains could potentially regulate transcription factor activity in these cells (e.g. in modulating p73 protein stability [117]). In this study, calpain-10 was expressed in small dot-like, dense sub-compartments of the nuclei of cortical lens fibre cells in two-week-old lenses, but this staining was lost by 12 weeks. Since nuclear structure and function are regulated by proteolysis in compartments of the nucleus [118], the role of calpains in this process during lens fibre cell differentiation therefore deserves attention.

A functional link between calpains and lens fibre cell differentiation was provided by studies in which p94 calpain was knocked out and normal maturational proteolysis in the lens was affected [119]. Lp82-mediated proteolysis also has a role in lens fibre cell differentiation [120,121], and sustained elevation in calcium could regulate lens cell differentiation, through triggering calpain activation [122]. This could be achieved through modulation of gap junction permeability [123,124].

There is recent evidence for an involvement of calpains in lens fibre cell differentiation. In calpain-3 knockout mice, it was observed that the cellular organization of the transition zone is altered, suggesting a calpain-3-dependent change in the lens fibre cell differentiation programme [113]. Calpain-1, -2, -3 and -7 were all expressed in lens fibre cells, with calpain-3 expressed strongly in the deep cortical fibre cells [125]. In this study, calpain-dependent cleavage of spectrin occurred late in lens fibre cell differentiation, and this coincided with lens fibre cell organelle degradation at the boundary region of the OFZ, suggesting that calpain activation in these cells could be a direct trigger for lens fibre cell organelle degradation. However, it could not be ruled out that calpain activation could be a consequence, rather than a cause, of organelle degradation owing to the release of calcium from degrading intracellular organelles, such as the endoplasmic reticulum and mitochondria.

### (d) *The ubiquitin–proteasome pathway*

The UPP is used to selectively degrade proteins [126] and is, for example, involved in degrading calpain-mediated C-terminal cleavage products of αA-crystallins during ageing and cataractogenesis [127]. The UPP acts through the attachment of ubiquitin to proteins in order to signal subsequent degradation of the protein by the 26S proteasome [126]. The lens possesses the UPP [128,129]. UPP activity increases in the equatorial region of the lens in which lens epithelial cells are beginning to differentiate into lens fibre cells [130], and UPP components are localized to the nuclei of differentiating lens fibre cells and in both the cytoplasm and nuclei of lens epithelial cells [131].

The UPP has now been implicated in the removal of organelle components during lens fibre cell differentiation, specifically in the degradation of mitochondria [12]. Proteasome inhibition by lactacystin was associated with accumulation of ubiquitinated proteins and reversible opacification of the lens cortex during development. In lactacystin-injected eyes, the programmed degradation of succinate–ubiquinone oxidoreductase was inhibited in the central lens fibre cells, but, interestingly, mitochondrial disintegration itself was not prevented, suggesting that the initiation of mitochondrial degradation is dependent on an as-yet unknown factor. The results also demonstrated that the VEIDase activity, previously postulated to be due to caspase-6 [87], was actually due to the UPP.

## 6. FUTURE PROSPECTS

Transgenic approaches continue to be used in mice to investigate over-expression of genes in specific tissues, including the lens. In addition, because of problems with embryonic lethality and difficulties in interpreting the results of studies in which classic gene knockout experiments are carried out, lens-specific gene deletions are increasingly being performed. For example, Cre-loxP systems have been used for conditional mutation of specific genes that are active in the whole lens, lens fibre cells or the early lens placode [132–135].

One such study, using both transgenic and cre-lox approaches, investigated the role of nuclear receptor coactivator 6 (Ncoa6) in lens fibre cell differentiation, apoptosis and organelle loss [136]. Ncoa6 is a

co-activator of nuclear receptors and other transcription factors and it has an anti-apoptotic function. When a dominant-negative mutant of Ncoa6 was expressed in lens fibre cells, the transgenic lenses showed reduced growth and lens fibre cell differentiation defects. The lens fibre cells also entered a pro-apoptotic pathway in which the degradation of lens fibre cell nuclei and organelles was delayed, owing to both p53-independent and p53-dependent pathways. However, issues of whether the retention of nuclei was due to a delay in lens fibre cell differentiation in general or a specific effect on denucleation are not easily answered using this approach. It is possible that over-expression of the dominant-negative mutant Ncao6 brought about a non-specific stress response [54], which resulted in activation of the delayed apoptotic pathway in the lens fibre cells. Indeed, a more compelling argument of a role for Ncoa6 in lens fibre cell differentiation and/or organelle loss was revealed using the cre-lox approach [136]. Lens-specific deletion of this pro-apoptotic gene resulted in defects in lens fibre cell differentiation and a delay in denucleation. The further development of lens-specific cre-lox systems using lens epithelial cell, lens fibre cell or even lens equatorial-specific gene promoters will further allow for the elucidation of specific gene functions in specific lens compartments pertaining to organelle loss during lens fibre cell differentiation.

Finally, the zebrafish will continue to provide a useful model system to investigate organelle loss during lens fibre cell differentiation. Despite the recognized morphological/developmental differences between zebrafish and other vertebrates with regard to lens development as outlined above, the zebrafish has many advantages, including the number of mutants available through forward genetic screens and the ease of embryo manipulation [7].

For example, recently, the zebrafish lens mutant *bumper* has been analysed [137]. In the mutant fish, there is a strong defect in secondary lens fibre cell differentiation (primary lens fibre cell differentiation appears to be unaffected), indicating that the bumper mutant could be an important model for examining the nature of the differences in the components of the molecular pathways of differentiation in primary versus secondary lens fibre cells. The *bumper* mutation was associated with the retention of lens fibre cell nuclei of normal appearance at 4 days post-fertilization beyond the stage when nuclei would normally be degenerating in the lens fibre cells. The authors suggested that there was a general defect in secondary lens fibre cell differentiation in the *bumper* mutant.

Another mutant zebrafish line that has recently proved very useful regarding lens fibre cell differentiation is the *volvox* (*vol*) mutant [138]. *Vol* encodes a component of the 26S proteasome; therefore, the mutants have a reduction in proteasome activity. *Vol* mutant fishes have defects in lens epithelial cell proliferation and lens fibre cell differentiation, including defects in the position, degradation and shape of lens fibre cell nuclei. This study again highlights the importance of the ubiquitin–proteasome system in lens fibre cell differentiation and lens fibre cell denucleation. No doubt additional zebrafish mutants will provide other important insights into these processes.

## 7. CONCLUSIONS

Thus, in conclusion, the study of the programmed loss of organelles to form an OFZ in the differentiating lens fibre cells has a long scientific history going back well over 100 years. Formation of the OFZ is of the utmost importance for the development and maintenance of transparency of the lens; defects in this process result in certain types of cataract in both humans and animal models. The molecular mechanisms involved in this process are being worked out and great progress has been made, but it is often unclear whether the effects observed are a direct result of defects in lens fibre cell organelle loss or whether they are actually indirect, owing to more general defects in lens fibre cell differentiation. The ongoing approaches being used, including biochemical techniques, transgenics and lens-specific gene deletion and gene knockout mice as well as examination of zebrafish mutants, will all shed light on this interesting phenomenon and will also lead to the dissection of the molecular pathways that contribute to it.

Given the evolutionary importance of clear lenses for vision across many species, it is likely that there are multiple redundant and parallel pathways acting in interacting networks during lens fibre cell differentiation, denucleation and organelle loss to varying degrees in different species. Indeed, it is clear from the work reviewed here that both tightly regulated apoptosis signalling pathways (without activating full classical apoptosis) and multiple proteolytic pathways have roles in regulating this process. Therefore, systems biology approaches focused, for example, on the apoptosis signalling and proteolysis networks in the lens may help to shed light on the way in which these networks contribute to the transparency of this unique and interesting tissue.

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## REFERENCES

1 Kuszak, J. R., Zoltoski, R. K. & Sivertson, C. 2004 Fibre cell organization in crystalline lenses. *Exp. Eye Res.* **78**, 673–687. (doi:10.1016/j.exer.2003.09.016)
2 Bassnett, S., Shi, Y. & Vrensen, G. F. J. M. 2011 Biological glass: structural determinants of eye lens transparency. *Phil. Trans. R. Soc. B* **366**, 1250–1264. (doi:10.1098/rstb.2010.0302)
3 Graw, J. 2009 Genetics of crystallins: cataract and beyond. *Exp. Eye Res.* **88**, 173–189. (doi:10.1016/j.exer.2008.10.011)
4 Vrensen, G. F., Graw, J. & De Wolf, A. 1991 Nuclear breakdown during terminal differentiation of primary lens fibres in mice: a transmission electron microscopic study. *Exp. Eye Res.* **52**, 647–659. (doi:10.1016/0014-4835(91)90017-9)
5 Ito, M. & Yoshioka, M. 1999 Regression of the hyaloid vessels and pupillary membrane of the mouse. *Anat. Embryol. (Berl.)* **200**, 403–411. (doi:10.1007/s004290050289)

6 Wride, M. A., Parker, E. & Sanders, E. J. 1999 Members of the bcl-2 and caspase families regulate nuclear degeneration during chick lens fibre differentiation. *Dev. Biol.* **213**, 142–156. (doi:10.1006/dbio.1999.9375)

7 Dahm, R., Schonthaler, H. B., Soehn, A. S., van Marle, J. & Vrensen, G. F. 2007 Development and adult morphology of the eye lens in the zebrafish. *Exp. Eye Res.* **85**, 74–89. (doi:10.1016/j.exer.2007.02.015)

8 Bassnett, S. 2009 On the mechanism of organelle degradation in the vertebrate lens. *Exp. Eye Res.* **88**, 133–139. (doi:10.1016/j.exer.2008.08.017)

9 Wride, M. A. 1996 Cellular and molecular features of lens differentiation: a review of recent advances. *Differentiation* **61**, 77–93. (doi:10.1046/j.1432-0436.1996.6120077.x)

10 Bassnett, S. & Mataic, D. 1997 Chromatin degradation in differentiating fiber cells of the eye lens. *J. Cell Biol.* **137**, 37–49. (doi:10.1083/jcb.137.1.37)

11 Wride, M. A. 2000 Minireview: apoptosis as seen through a lens. *Apoptosis* **5**, 203–209. (doi:10.1023/A:1009653326511)

12 Zandy, A. J. & Bassnett, S. 2007 Proteolytic mechanisms underlying mitochondrial degradation in the ocular lens. *Invest. Ophthalmol. Vis. Sci.* **48**, 293–302. (doi:10.1167/iovs.06-0656)

13 McAvoy, J. W., Palfrey, L. J. & van Heyningen, R. 1979 A light microscope study of the lens of the tryptophan deficient rat. *Exp. Eye Res.* **28**, 533–538. (doi:10.1016/0014-4835(79)90041-1)

14 Wegener, A., Golubnitschaja, O., Breipohl, W., Schild, H. H. & Vrensen, G. F. 2002 Effects of dietary deficiency of selective amino acids on the function of the cornea and lens in rats. *Amino Acids* **23**, 337–342. (doi:10.1007/s00726-001-0147-x)

15 Vrensen, G. F., van Marle, J., Jonges, R., Voorhout, W., Breipohl, W. & Wegener, A. R. 2004 Tryptophan deficiency arrests chromatin breakdown in secondary lens fibers of rats. *Exp. Eye Res.* **78**, 661–672. (doi:10.1016/j.exer.2003.07.004)

16 Lockshin, R. A. & Zakeri, Z. 2004 Caspase-independent cell death? *Oncogene* **23**, 2766–2773. (doi:10.1038/sj.onc.1207514)

17 Oparka, K. J. & Turgeon, R. 1999 Sieve elements and companion cells—traffic control centers of the phloem. *Plant Cell* **11**, 739–750. (doi:10.1105/tpc.11.4.739)

18 Dahm, R. 2004 Dying to see. *Sci. Am.* **291**, 82–89. (doi:10.1038/scientificamerican1004-82)

19 Counis, M. F., Chaudun, E., Allinquant, B., Muel, A. S., Sanval, M., Skidmore, C. & Courtois, Y. 1989 The lens: a model for chromatin degradation studies in terminally differentiating cells. *Int. J. Biochem.* **21**, 235–242. (doi:10.1016/0020-711X(89)90181-X)

20 Bassnett, S. 2002 Lens organelle degradation. *Exp. Eye Res.* **74**, 1–6. (doi:10.1006/exer.2001.1111)

21 Dahm, R. 1999 Lens fibre cell differentiation—a link with apoptosis? *Ophthal. Res.* **31**, 163–183. (doi:10.1159/000055530)

22 Wride, M. A., Geatrell, J. & Guggenheim, J. A. 2006 Proteases in eye development and disease. *Birth Defects Res. C Embryo Today* **78**, 90–105. (doi:10.1002/bdrc.20063)

23 Wride, M. A. 2007 Role of proteases in development and diseases of the lens. *Exp. Rev. Ophthalmol.* **2**, 833–844. (doi:10.1586/17469899.2.5.833)

24 Dahm, R. 2009 Nuclear degradation in the lens circa 1897–1899. *Scientist* **23**, 84.

25 Rabl, C. 1899 Uber den bau und die entwicklung der linse: III Teil:die lines der saugethiere: Ruckblick und Schluss. *Z. Wiss. Zool.* **67**, 1–138.

26 Coulombre, J. L. & Coulombre, A. J. 1963 Lens development: fiber elongation and lens orientation. *Science* **142**, 1489–1490. (doi:10.1126/science.142.3598.1489)

27 Modak, S. P. & Perdue, S. W. 1970 Terminal lens cell differentiation. I. Histological and microspectrophotometric analysis of nuclear degeneration. *Exp. Cell Res.* **59**, 43–56. (doi:10.1016/0014-4827(70)90622-1)

28 Modak, S. P., von Borstel, R. C. & Bollum, F. J. 1969 Terminal lens cell differentiation. II. Template activity of DNA during nuclear degeneration. *Exp. Cell Res.* **56**, 105–113. (doi:10.1016/0014-4827(69)90401-7)

29 Modak, S. P. & Bollum, F. J. 1970 Terminal lens cell differentiation. III. Initiator activity of DNA during nuclear degeneration. *Exp. Cell Res.* **62**, 421–432. (doi:10.1016/0014-4827(70)90573-2)

30 Modak, S. P. & Bollum, F. J. 1972 Detection and measurement of single-strand breaks in nuclear DNA in fixed lens sections. *Exp. Cell Res.* **75**, 307–313. (doi:10.1016/0014-4827(72)90434-X)

31 Gavrieli, Y., Sherman, Y. & Ben-Sasson, S. A. 1992 Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.* **119**, 493–501. (doi:10.1083/jcb.119.3.493)

32 Appleby, D. W. & Modak, S. P. 1977 DNA degradation in terminally differentiating lens fiber cells from chick embryos. *Proc. Natl Acad. Sci. USA* **74**, 5579–5583. (doi:10.1073/pnas.74.12.5579)

33 Kuwabara, T. & Imaizumi, M. 1974 Denucleation process of the lens. *Invest. Ophthalmol.* **13**, 973–981.

34 Hamai, Y., Fukui, H. N. & Kuwabara, T. 1974 Morphology of hereditary mouse cataract. *Exp. Eye Res.* **18**, 537–546. (doi:10.1016/0014-4835(74)90060-8)

35 Pendergrass, W., Penn, P., Possin, D. & Wolf, N. 2005 Accumulation of DNA, nuclear and mitochondrial debris, and ROS at sites of age-related cortical cataract in mice. *Invest. Ophthalmol. Vis. Sci.* **46**, 4661–4670. (doi:10.1167/iovs.05-0808)

36 Pendergrass, W. R., Penn, P. E., Possin, D. E. & Wolf, N. S. 2006 Cellular debris and ROS in age-related cortical cataract are caused by inappropriate involution of the surface epithelial cells into the lens cortex. *Mol. Vis.* **12**, 712–724.

37 McAlister Gregg, N. 2001 Congenital cataract following German measles in the mother. (1942, classical article). *Rev. Med. Virol.* **11**, 277–283. Discussion 84–5. (doi:10.1002/rmv.327)

38 Webster, W. S. 1998 Teratogen update: congenital rubella. *Teratology* **58**, 13–23. (doi:10.1002/(SICI)1096-9926(199807)58:1<13::AID-TERA5>3.0.CO;2-2)

39 Piatigorsky, J., Rothschild, S. S. & Milstone, L. M. 1973 Differentiation of lens fibers in explanted embryonic chick lens epithelia. *Dev. Biol.* **34**, 334–345. (doi:10.1016/0012-1606(73)90362-X)

40 McAvoy, J. W. & Richardson, N. A. 1986 Nuclear pyknosis during lens fibre differentiation in epithelial explants. *Curr. Eye Res.* **5**, 711–715. (doi:10.3109/02713688609015139)

41 Menko, A. S., Klukas, K. A. & Johnson, R. G. 1984 Chicken embryo lens cultures mimic differentiation in the lens. *Dev. Biol.* **103**, 129–141. (doi:10.1016/0012-1606(84)90014-9)

42 Wride, M. A. & Sanders, E. J. 1998 Nuclear degeneration in the developing lens and its regulation by TNFα. *Exp. Eye Res.* **66**, 371–383. (doi:10.1006/exer.1997.0440)

43 Marcantonio, J. M. & Vrensen, G. F. 1999 Cell biology of posterior capsular opacification. *Eye (Lond.)* **13**, 484–488.

44 Nagata, S. 2005 DNA degradation in development and programmed cell death. *Annu. Rev. Immunol.* **23**, 853–875. (doi:10.1146/annurev.immunol.23.021704.115811)

45 Bassnett, S. & Beebe, D. C. 1992 Coincident loss of mitochondria and nuclei during lens fiber cell differentiation. *Dev. Dyn.* **194**, 85–93.

46 Li, J. & Yuan, J. 2008 Caspases in apoptosis and beyond. *Oncogene* **27**, 6194–6206. (doi:10.1038/onc.2008.297)

47 Zandy, A. J., Lakhani, S., Zheng, T., Flavell, R. A. & Bassnett, S. 2005 Role of the executioner caspases during lens development. *J. Biol. Chem.* **280**, 30 263–30 272. (doi:10.1074/jbc.M504007200)

48 Kestler, H. A., Wawra, C., Kracher, B. & Kuhl, M. 2008 Network modeling of signal transduction: establishing the global view. *Bioessays* **30**, 1110–1125. (doi:10.1002/bies.20834)

49 Lachke, S. A. & Maas, R. L. 2010 Building the developmental oculome: systems biology in vertebrate eye development and disease. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2**, 305–323. (doi:10.1002/wsbm.59)

50 Rao, T. P. & Kuhl, M. 2010 An updated overview on Wnt signaling pathways: a prelude for more. *Circ. Res.* **106**, 1798–1806. (doi:10.1161/CIRCRESAHA.110.219840)

51 Medina-Martinez, O. & Jamrich, M. 2007 Foxe view of lens development and disease. *Development* **134**, 1455–1463. (doi:10.1242/dev.000117)

52 Lavrik, I. N., Eils, R., Fricker, N., Pforr, C. & Krammer, P. H. 2009 Understanding apoptosis by systems biology approaches. *Mol. Biosyst.* **5**, 1105–1111. (doi:10.1039/b905129p)

53 Jung, T., Catalgol, B. & Grune, T. 2009 The proteasomal system. *Mol. Aspects Med.* **30**, 191–296. (doi:10.1016/j.mam.2009.04.001)

54 Firtina, Z., Danysh, B. P., Bai, X., Gould, D. B., Kobayashi, T. & Duncan, M. K. 2009 Abnormal expression of collagen IV in lens activates unfolded protein response resulting in cataract. *J. Biol. Chem.* **284**, 35 872–35 884. (doi:10.1074/jbc.M109.060384)

55 Greiling, T. M. & Clark, J. I. 2009 Early lens development in the zebrafish: a three-dimensional time-lapse analysis. *Dev. Dyn.* **238**, 2254–2265. (doi:10.1002/dvdy.21997)

56 Wride, M. A., Mansergh, F. C., Adams, S., Everitt, R., Minnema, S. E., Rancourt, D. E. & Evans, M. J. 2003 Expression profiling and gene discovery in the mouse lens. *Mol. Vis.* **9**, 360–396.

57 Geatrell, J. C., Gan, P. M., Mansergh, F. C., Kisiswa, L., Jarrin, M., Williams, L. A., Evans, M. J., Boulton, M. E. & Wride, M. A. 2009 Apoptosis gene profiling reveals spatio-temporal regulated expression of the p53/Mdm2 pathway during lens development. *Exp. Eye Res.* **88**, 1137–1151. (doi:10.1016/j.exer.2009.01.020)

58 Weber, G. F. & Menko, A. S. 2005 The canonical intrinsic mitochondrial death pathway has a non-apoptotic role in signaling lens cell differentiation. *J. Biol. Chem.* **280**, 22 135–22 145. (doi:10.1074/jbc.M414270200)

59 Sanders, E. J. & Parker, E. 2003 Retroviral overexpression of bcl-2 in the embryonic chick lens influences denucleation in differentiating lens fiber cells. *Differentiation* **71**, 425–433. (doi:10.1046/j.1432-0436.2003.7107005.x)

60 Fromm, L. & Overbeek, P. A. 1997 Inhibition of cell death by lens-specific overexpression of bcl-2 in transgenic mice. *Dev. Genet.* **20**, 276–287. (doi:10.1002/(SICI)1520-6408(1997)20:3<276::AID-DVG10>3.0.CO;2-6)

61 Williams, L. 2008 *The expression of tumour necrosis factors during chick lens development*. Cardiff, Wales, UK: Cardiff University.

62 Bassnett, S. & McNulty, R. 2003 The effect of elevated intraocular oxygen on organelle degradation in the embryonic chicken lens. *J. Exp. Biol.* **206**, 4353–4361. (doi:10.1242/jeb.00670)

63 Landgren, H., Blixt, A. & Carlsson, P. 2008 Persistent FoxE3 expression blocks cytoskeletal remodeling and organelle degradation during lens fiber differentiation. *Invest. Ophthalmol. Vis. Sci.* **49**, 4269–4277. (doi:10.1167/iovs.08-2243)

64 Loren, C. E., Schrader, J. W., Ahlgren, U. & Gunhaga, L. 2009 FGF signals induce Caprin2 expression in the vertebrate lens. *Differentiation* **77**, 386–394. (doi:10.1016/j.diff.2008.11.003)

65 Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery Jr, C. A., Butel, J. S. & Bradley, A. 1992 Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215–221. (doi:10.1038/356215a0)

66 Sah, V. P., Attardi, L. D., Mulligan, G. J., Williams, B. O., Bronson, R. T. & Jacks, T. 1995 A subset of p53-deficient embryos exhibit exencephaly. *Nat. Genet.* **10**, 175–180. (doi:10.1038/ng0695-175)

67 Ikeda, S., Hawes, N. L., Chang, B., Avery, C. S., Smith, R. S. & Nishina, P. M. 1999 Severe ocular abnormalities in C57BL/6 but not in 129/Sv p53-deficient mice. *Invest. Ophthalmol. Vis. Sci.* **40**, 1874–1878.

68 Pokroy, R., Tendler, Y., Pollack, A., Zinder, O. & Weisinger, G. 2002 p53 expression in the normal murine eye. *Invest. Ophthalmol. Vis. Sci.* **43**, 1736–1741.

69 Ayala, M., Strid, H., Jacobsson, U. & Soderberg, P. G. 2007 p53 expression and apoptosis in the lens after ultraviolet radiation exposure. *Invest. Ophthalmol. Vis. Sci.* **48**, 4187–4191. (doi:10.1167/iovs.06-0339)

70 Pan, H. & Griep, A. E. 1995 Temporally distinct patterns of p53-dependent and p53-independent apoptosis during mouse lens development. *Genes Dev.* **9**, 2157–2169. (doi:10.1101/gad.9.17.2157)

71 Nakamura, T., Pichel, J. G., Williams-Simons, L. & Westphal, H. 1995 An apoptotic defect in lens differentiation caused by human p53 is rescued by a mutant allele. *Proc. Natl Acad. Sci. USA* **92**, 6142–6146. (doi:10.1073/pnas.92.13.6142)

72 Peller, S. *et al.* 2003 The onset of p53-dependent apoptosis plays a role in terminal differentiation of human normoblasts. *Oncogene* **22**, 4648–4655. (doi:10.1038/sj.onc.1206541)

73 Maetens, M., Doumont, G., Clercq, S. D., Francoz, S., Froment, P., Bellefroid, E., Klingmuller, U., Lozano, G. & Marine, J.-C. 2007 Distinct roles of Mdm2 and Mdm4 in red cell production. *Blood* **109**, 2630–2633. (doi:10.1182/blood-2006-03-013656)

74 Weber, G. F. & Menko, A. S. 2006 Phosphatidylinositol 3-kinase is necessary for lens fiber cell differentiation and survival. *Invest. Ophthalmol. Vis. Sci.* **47**, 4490–4499. (doi:10.1167/iovs.06-0401)

75 Mansergh, F. C., Wride, M. A., Walker, V. E., Adams, S., Hunter, S. M. & Evans, M. J. 2004 Gene expression changes during cataract progression in Sparc null mice: differential regulation of mouse globins in the lens. *Mol. Vis.* **10**, 490–511.

76 Mansergh, F. C., Hunter, S. M., Geatrell, J. C., Jarrin, M., Powell, K., Evans, M. J. & Wride, M. A. 2008 Developmentally regulated expression of hemoglobin subunits in avascular tissues. *Int. J. Dev. Biol.* **52**, 873–886. (doi:10.1387/ijdb.082597fm)

77 Brecht, K., Simonen, M. & Heim, J. 2005 Upregulation of alpha globin promotes apoptotic cell death in the hematopoietic cell line FL5.12. *Apoptosis* **10**, 1043–1062. (doi:10.1007/s10495-005-0617-9)

78 Brecht, K., Simonen, M., Kamke, M. & Heim, J. 2005 Hematopoietic transcription factor GATA-2 promotes upregulation of alpha globin and cell death in FL5.12 cells. *Apoptosis* **10**, 1063–1078. (doi:10.1007/s10495-005-0623-y)

79 Matsui, M., Yamamoto, A., Kuma, A., Ohsumi, Y. & Mizushima, N. 2006 Organelle degradation during the lens and erythroid differentiation is independent of autophagy. *Biochem. Biophys. Res. Commun.* **339**, 485–489. (doi:10.1016/j.bbrc.2005.11.044)

80 Ishizaki, Y., Jacobson, M. D. & Raff, M. C. 1998 A role for caspases in lens fiber differentiation. *J. Cell Biol.* **140**, 153–158. (doi:10.1083/jcb.140.1.153)

81 Sanders, E. J. & Parker, E. 2002 The role of mitochondria, cytochrome c and caspase-9 in embryonic lens fibre cell denucleation. *J. Anat.* **201**, 121–135. (doi:10.1046/j.1469-7580.2002.00081.x)

82 Weber, G. F. & Menko, A. S. 2006 Actin filament organization regulates the induction of lens cell differentiation and survival. *Dev. Biol.* **295**, 714–729. (doi:10.1016/j.ydbio.2006.03.056)

83 Wei, C. J., Xu, X. & Lo, C. W. 2004 Connexins and cell signaling in development and disease. *Annu. Rev. Cell Dev. Biol.* **20**, 811–838. (doi:10.1146/annurev.cellbio.19.111301.144309)

84 Yin, X., Gu, S. & Jiang, J. X. 2001 Regulation of lens connexin 45.6 by apoptotic protease, caspase-3. *Cell Commun. Adhes.* **8**, 373–376. (doi:10.3109/154190 60109080756)

85 Yin, X., Gu, S. & Jiang, J. X. 2001 The development-associated cleavage of lens connexin 45.6 by caspase-3-like protease is regulated by casein kinase II-mediated phosphorylation. *J. Biol. Chem.* **276**, 34 567–34 572. (doi:10.1074/jbc.M106073200)

86 Lee, A., Morrow, J. S. & Fowler, V. M. 2001 Caspase remodeling of the spectrin membrane skeleton during lens development and aging. *J. Biol. Chem.* **276**, 20 735–20 742. (doi:10.1074/jbc.M009723200)

87 Foley, J. D., Rosenbaum, H. & Griep, A. E. 2004 Temporal regulation of VEID-7-amino-4-trifluoromethylcoumarin cleavage activity and caspase-6 correlates with organelle loss during lens development. *J. Biol. Chem.* **279**, 32 142–32 150. (doi:10.1074/jbc.M313683200)

88 Morozov, V. & Wawrousek, E. F. 2006 Caspase-dependent secondary lens fiber cell disintegration in αA-/αB-crystallin double-knockout mice. *Development* **133**, 813–821. (doi:10.1242/dev.02262)

89 Strickler, A. G., Byerly, M. S. & Jeffery, W. R. 2007 Lens gene expression analysis reveals downregulation of the anti-apoptotic chaperone αA-crystallin during cavefish eye degeneration. *Dev. Genes Evol.* **217**, 771–782. (doi:10.1007/s00427-007-0190-z)

90 Yamashita, M., Mizusawa, N., Hojo, M. & Yabu, T. 2008 Extensive apoptosis and abnormal morphogenesis in procaspase-3 transgenic zebrafish during development. *J. Exp. Biol.* **211**, 1874–1881. (doi:10.1242/jeb.012690)

91 Ruiz-Vela, A., Gonzalez de Buitrago, G. & Martinez, A. C. 2002 Nuclear Apaf-1 and cytochrome c redistribution following stress-induced apoptosis. *FEBS Lett.* **517**, 133–138. (doi:10.1016/S0014-5793 (02)02607-8)

92 Cecconi, F., Alvarez-Bolado, G., Meyer, B. I., Roth, K. A. & Gruss, P. 1998 Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. *J. Cell* **94**, 727–737.

93 Zermati, Y. *et al.* 2007 Nonapoptotic role for Apaf-1 in the DNA damage checkpoint. *Mol. Cell* **28**, 624–637. (doi:10.1016/j.molcel.2007.09.030)

94 Chaudun, E., Arruti, C., Courtois, Y., Ferrag, F., Jeanny, J. C., Patel, B. N., Skidmore, C., Torriclia, A. & Counis, M. F. 1994 DNA strand breakage during physiological apoptosis of the embryonic chick lens: free 3′ OH end single strand breaks do not accumulate even in the presence of a cation-independent deoxyribonuclease. *J. Cell Physiol.* **158**, 354–364. (doi:10.1002/jcp.1041580218)

95 Counis, M. F., Chaudun, E., Simonneau, L. & Courtois, Y. 1979 DNA repair in lens cells during chick embryo development. *Biochim. Biophys. Acta* **561**, 85–98.

96 Yang, Y. G., Frappart, P. O., Frappart, L., Wang, Z. Q. & Tong, W. M. 2006 A novel function of DNA repair molecule Nbs1 in terminal differentiation of the lens fibre cells and cataractogenesis. *DNA Repair* (*Amst.*) **5**, 885–893.

97 Vakifahmetoglu-Norberg, H. & Zhivotovsky, B. 2010 The unpredictable caspase-2: what can it do? *Trends Cell Biol.* **20**, 150–159. (doi:10.1016/j.tcb.2009.12.006)

98 Chwieralski, C. E., Welte, T. & Buhling, F. 2006 Cathepsin-regulated apoptosis. *Apoptosis* **11**, 143–149. (doi:10.1007/s10495-006-3486-y)

99 Zuzarte-Luis, V., Montero, J. A., Kawakami, Y., Izpisua-Belmonte, J. C. & Hurle, J. M. 2007 Lysosomal cathepsins in embryonic programmed cell death. *Dev. Biol.* **301**, 205–217. (doi:10.1016/j.ydbio.2006.08.008)

100 Zuzarte-Luis, V., Montero, J. A., Torre-Perez, N., Garcia-Porrero, J. A. & Hurle, J. M. 2007 Cathepsin D gene expression outlines the areas of physiological cell death during embryonic development. *Dev. Dyn.* **236**, 880–885. (doi:10.1002/dvdy.21076)

101 Im, E. & Kazlauskas, A. 2007 The role of cathepsins in ocular physiology and pathology. *Exp. Eye Res.* **84**, 383–388. (doi:10.1016/j.exer.2006.05.017)

102 Nishimoto, S. *et al.* 2003 Nuclear cataract caused by a lack of DNA degradation in the mouse eye lens. *Nature* **424**, 1071–1074. (doi:10.1038/nature01895)

103 De Maria, A. & Bassnett, S. 2007 DNaseII beta distribution and activity in the mouse lens. *Invest. Ophthalmol. Vis. Sci.* **48**, 5638–5646. (doi:10.1167/iovs.07-0782)

104 Nakahara, M., Nagasaka, A., Koike, M., Uchida, K., Kawane, K., Uchiyama, Y. & Nagata, S. 2007 Degradation of nuclear DNA by DNase II-like acid DNase in cortical fiber cells of mouse eye lens. *FEBS J.* **274**, 3055–3064. (doi:10.1111/j.1742-4658.2007.05836.x)

105 Hirano, M. *et al.* 2003 Generation of structures formed by lens and retinal cells differentiating from embryonic stem cells. *Dev. Dyn.* **228**, 664–671. (doi:10.1002/dvdy.10425)

106 Aoki, H., Hara, A., Niwa, M., Motohashi, T., Suzuki, T. & Kunisada, T. 2008 Transplantation of cells from eye-like structures differentiated from embryonic stem cells *in vitro* and *in vivo* regeneration of retinal ganglion-like cells. *Graefes Arch. Clin. Exp. Ophthalmol.* **246**, 255–265. (doi:10.1007/s00417-007-0710-6)

107 Yang, C., Yang, Y., Brennan, L., Bouhassira, E. E., Kantorow, M. & Cvekl, A. 2010 Efficient generation of lens progenitor cells and lentoid bodies from human embryonic stem cells in chemically defined conditions. *Faseb J* **24**, 3274–3283. (doi:10.1096/fj.10-157255)

108 Shearer, T. R., Ma, H., Shih, M., Fukiage, C. & Azuma, M. 2000 Calpains in the lens and cataractogenesis. *Methods Mol. Biol.* **144**, 277–285.

109 Biswas, S., Harris, F., Dennison, S., Singh, J. & Phoenix, D. A. 2004 Calpains: targets of cataract prevention? *Trends Mol. Med.* **10**, 78–84. (doi:10.1016/j.molmed.2003.12.007)

110 Biswas, S., Harris, F., Dennison, S., Singh, J. P. & Phoenix, D. 2005 Calpains: enzymes of vision? *Med. Sci. Monit.* **11**, RA301–RA310.

111 Ma, H., Azuma, M. & Shearer, T. R. 2005 Degradation of human aquaporin 0 by m-calpain. *FEBS Lett.* **579**, 6745–6748. (doi:10.1016/j.febslet.2005.11.004)

112 Shih, M., Ma, H., Nakajima, E., David, L. L., Azuma, M. & Shearer, T. R. 2006 Biochemical properties of lens-specific calpain Lp85. *Exp. Eye Res.* **82**, 146–152. (doi:10.1016/j.exer.2005.06.011)

113 Tang, Y., Liu, X., Zoltoski, R. K., Novak, L. A., Herrera, R. A., Richard, I., Kuszak, J. R. & Kumar, N. M. 2007 Age-related cataracts in α3Cx46-knockout mice are dependent on a calpain 3 isoform. *Invest. Ophthalmol. Vis. Sci.* **48**, 2685–2694. (doi:10.1167/iovs.06-0926)

114 Elanchezhian, R., Sakthivel, M., Geraldine, P. & Thomas, P. A. 2009 The effect of acetyl-L-carnitine on lenticular calpain activity in prevention of selenite-induced cataractogenesis. *Exp. Eye Res.* **88**, 938–944. (doi:10.1016/j.exer.2008.12.009)

115 Lee, H. Y., Morton, J. D., Robertson, L. J., McDermott, J. D., Bickerstaffe, R., Abell, A. D., Jones, M. A., Mehrtens, J. M. & Coxon, J. M. 2008 Evaluation of a novel calpain inhibitor as a treatment for cataract. *Clin. Exp. Ophthalmol.* **36**, 852–860. (doi:10.1111/j.1442-9071.2009.01925.x)

116 Reed, N. A., Castellini, M. A., Ma, H., Shearer, T. R. & Duncan, M. K. 2003 Protein expression patterns for ubiquitous and tissue specific calpains in the developing mouse lens. *Exp. Eye Res.* **76**, 433–443. (doi:10.1016/S0014-4835(02)00336-6)

117 Munarriz, E., Bano, D., Sayan, A. E., Rossi, M., Melino, G. & Nicotera, P. 2005 Calpain cleavage regulates the protein stability of p73. *Biochem. Biophys. Res. Commun.* **333**, 954–960. (doi:10.1016/j.bbrc.2005.05.188)

118 Mijaljica, D., Prescott, M. & Devenish, R. J. 2007 Nibbling within the nucleus: turnover of nuclear contents. *Cell. Mol. Life Sci.* **64**, 581–588. (doi:10.1007/s00018-007-6395-5)

119 Ma, H., Shih, M., Fukiage, C., Azuma, M., Duncan, M. K., Reed, N. A., Richard, I., Beckmann, J. S. & Shearer, T. R. 2000 Influence of specific regions in Lp82 calpain on protein stability, activity, and localization within lens. *Invest. Ophthalmol. Vis. Sci.* **41**, 4232–4239.

120 Azuma, M., Tamada, Y., Kanaami, S., Nakajima, E., Nakamura, Y., Fukiage, C., Forsberg, N. E., Duncan, M. K. & Shearer, T. R. 2003 Differential influence of proteolysis by calpain 2 and Lp82 on *in vitro* precipitation of mouse lens crystallins. *Biochem. Biophys. Res. Commun.* **307**, 558–563. (doi:10.1016/S0006-291X(03)01194-X)

121 Inomata, M., Hayashi, M., Ito, Y., Matsubara, Y., Takehana, M., Kawashima, S. & Shumiya, S. 2002 Comparison of Lp82- and m-calpain-mediated proteolysis during cataractogenesis in Shumiya cataract rat (SCR). *Curr. Eye Res.* **25**, 207–213. (doi:10.1076/ceyr.25.4.207.13486)

122 Churchill, G. C. & Louis, C. F. 2002 Ca(2+) regulation in differentiating lens cells in culture. *Exp. Eye Res.* **75**, 77–85. (doi:10.1006/exer.2002.1184)

123 Baruch, A., Greenbaum, D., Levy, E. T., Nielsen, P. A., Gilula, N. B., Kumar, N. M. & Bogyo, M. 2001 Defining a link between gap junction communication, proteolysis, and cataract formation. *J. Biol. Chem.* **276**, 28 999–29 006. (doi:10.1074/jbc.M103628200)

124 Gao, J., Sun, X., Martinez-Wittinghan, F. J., Gong, X., White, T. W. & Mathias, R. T. 2004 Connections between connexins, calcium, and cataracts in the lens. *J. Gen. Physiol.* **124**, 289–300. (doi:10.1085/jgp.200409121)

125 De Maria, A., Shi, Y., Kumar, N. M. & Bassnett, S. 2009 Calpain expression and activity during lens fiber cell differentiation. *J. Biol. Chem.* **284**, 13542–13550. (doi:10.1074/jbc.M900561200)

126 Varshavsky, A. 2005 Regulated protein degradation. *Trends Biochem. Sci.* **30**, 283–286. (doi:10.1016/j.tibs.2005.04.005)

127 Zhang, X., Dudek, E. J., Liu, B., Ding, L., Fernandes, A. F., Liang, J. J., Horwitz, J., Taylor, A. & Shang, F. 2007 Degradation of C-terminal truncated αA-crystallins by the ubiquitin-proteasome pathway. *Invest. Ophthalmol. Vis. Sci.* **48**, 4200–4208. (doi:10.1167/iovs.07-0196)

128 Pereira, P., Shang, F., Hobbs, M., Girao, H. & Taylor, A. 2003 Lens fibers have a fully functional ubiquitin-proteasome pathway. *Exp. Eye Res.* **76**, 623–631. (doi:10.1016/S0014-4835(03)00020-4)

129 Shang, F., Gong, X., Palmer, H. J., Nowell Jr, T. R. & Taylor, A. 1997 Age-related decline in ubiquitin conjugation in response to oxidative stress in the lens. *Exp. Eye Res.* **64**, 21–30. (doi:10.1006/exer.1996.0176)

130 Shang, F., Gong, X., McAvoy, J. W., Chamberlain, C., Nowell Jr, T. R. & Taylor, A. 1999 Ubiquitin-dependent pathway is up-regulated in differentiating lens cells. *Exp. Eye Res.* **68**, 179–192. (doi:10.1006/exer.1998.0576)

131 Girao, H., Pereira, P., Taylor, A. & Shang, F. 2005 Subcellular redistribution of components of the ubiquitin-proteasome pathway during lens differentiation and maturation. *Invest. Ophthalmol. Vis. Sci.* **46**, 1386–1392. (doi:10.1167/iovs.04-0563)

132 Ashery-Padan, R., Marquardt, T., Zhou, X. & Gruss, P. 2000 Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. *Genes Dev.* **14**, 2701–2711. (doi:10.1101/gad.184000)

133 Cain, S., Martinez, G., Kokkinos, M. I., Turner, K., Richardson, R. J., Abud, H. E., Huelsken, J., Robinson, M. & Deiongh, R. 2008 Differential requirement for β-catenin in epithelial and fiber cells during lens development. *Dev. Biol.* **321**, 420–433. (doi:10.1016/j.ydbio.2008.07.002)

134 Martinez, G., Wijesinghe, M., Turner, K., Abud, H. E., Taketo, M. M., Noda, T., Robinson, M. L. & de Iongh, R. U. 2009 Conditional mutations of β-catenin and APC reveal roles for canonical Wnt signaling in lens differentiation. *Invest. Ophthalmol. Vis. Sci.* **50**, 4794–4806. (doi:10.1167/iovs.09-3567)

135 Shaham, O., Smith, A. N., Robinson, M. L., Taketo, M. M., Lang, R. A. & Ashery-Padan, R. 2009 Pax6 is essential for lens fiber cell differentiation. *Development* **136**, 2567–2578. (doi:10.1242/dev.032888)

136 Wang, W. L., Li, Q., Xu, J. & Cvekl, A. 2010 Lens fiber cell differentiation and denucleation are disrupted through expression of the N-terminal nuclear receptor box of Ncoa6 and result in p53-dependent and p53-independent apoptosis. *Mol. Biol. Cell* **21**, 2453–2468. (doi:10.1091/mbc.E09-12-1031)

137 Schonthaler, H. B., Franz-Odendaal, T. A., Hodel, C., Gehring, I., Geisler, R., Schwarz, H., Neuhauss, S. C. F. & Dahm, R. 2010 The zebrafish mutant bumper shows a hyperproliferation of lens epithelial cells and fibre cell degeneration leading to functional blindness. *Mech. Dev.* **127**, 203–219. (doi:10.1016/j.mod.2010.01.005)

138 Imai, F., Yoshizawa, A., Fujimori-Tonou, N., Kawakami, K. & Masai, I. 2010 The ubiquitin proteasome system is required for cell proliferation of the lens epithelium and for differentiation of lens fiber cells in zebrafish. *Development* **137**, 3257–3268. (doi:10.1242/dev.053124)