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Insights into the beaded filament of the eye lens

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Abstract

Filensin (BFSP1) and CP49 (BFSP2) represent two members of the IF protein superfamily that are thus far exclusively expressed in the eye lens. Mutations in both proteins cause lens cataract and careful consideration of the detail of these cataract phenotypes alerts us to several interesting features concerning the function of filensin (BFSP1) and CP49 (BFSP2) in the lens. With the first filensin (BFSP1) mutation now having been reported to cause a recessive cataract phenotype, there is the suggestion that the mutation could predispose heterozygote carriers to the early onset of age-related nuclear cataract. In the case of CP49 (BFSP2), there are now three unrelated families who have been identified with a common E233Δ mutation. Very interestingly this is linked to myopia in one family. Despite the apparent phenotypic differences of the filensin (BFSP1) and CP49 (BFSP2) mutations, the data are still consistent with the beaded filament proteins being essential for lens function and specifically contributing to the optical properties of the lens. The fact that none of the mutations thus far reported affect either the conserved LNDR or TYRKLLEGE motifs that flank the central rod domain supports the view that this pair of IF proteins have unusual structural features and a distinctive assembly mechanism. The multiple sequence divergences suggest these proteins have been adapted to the specific functional requirements of lens fibre cells, a function that can be traced from squid to man.

Introduction

The eye lens is a transparent tissue comprising highly elongated lens fibre cells, which are derived from the differentiation of a single layer of polarised epithelial cells that underlie the anterior surface of the lens (Fig. 1). The process of differentiation is accompanied by characteristic changes in the intracellular architecture of lens fibre cells. These include cellular elongation, the synthesis of certain crystallins [1, 2] and the loss of all the membrane-bound organelles including nuclei [3]. Despite these alterations, the differentiated lens fibre cells maintain a well-organised lenticular cytoskeleton comprising actin-containing microfilaments, microtubules and at least two different IF networks, one based on vimentin and the other based on a copolymer of lens-specific IF proteins [4].

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The lenticular IF cytoskeleton

The first identified IF protein in the eye lens is vimentin, a type III IF protein found in both the undifferentiated lens epithelium as well as the differentiated lens fibre cells [5, 6]. The other IF proteins found exclusively in the lens fibre cells [4, 7] comprise a unique cytoskeletal structure called the beaded filament [8]. Based on SDS-PAGE of the beaded filament fraction enriched from the chicken lens, the two structural proteins of the beaded filament were called cytoskeletal protein 49 (CP49) and cytoskeletal protein 95 (CP95) [9, 10]. The CP95 orthologues in the cow and rat lenses were called CP115 [11] and CP94 [12] respectively. To avoid confusion due to these different apparent molecular weights in the different species, this protein was renamed filensin [13, 14] or beaded filament structural protein 1 (BFSP1). CP49 has also been called phakinin [15] or beaded filament structural protein 2 (BFSP2), but it does not exhibit as much electrophoretic mobility variability between species. Unlike vimentin that is expressed in many cell types, filensin (BFSP1) and CP49 (BFSP2) are exclusively expressed in the fibre cells of the eye lens [4, 7].

Unique structural details of lens-specific IF proteins

Analysis of primary and secondary sequence of filensin (BFSP1) and CP49 (BFSP2) from several species confirmed that both proteins are members of IF protein family [14-19]. These two proteins, however, revealed several unique sequence characteristics, which do not fit easily into any of the currently established classes of IF proteins [20, 21].

Filensin (BFSP1) has a number of distinguishing features, such as a shortened rod domain resulting from a truncation of 29 amino acids within the helix 2 immediate after the fourth heptad repeat [14, 18]. This truncation means that filensin has the shortest rod domain of all cytoplasmic IF proteins. Filensin (BFSP1) also exhibits sequence divergence in both the highly conserved motifs at ends of the rod domain. Whilst the TYRKLLEGEE motif at the end of the rod domain has the modified sequence RYHRIIE(I/N)EG, the LNDR motif at the beginning of the rod domain is altered to LGER in mammalian filensin (BFSP1) [22].

CP49 (BFSP2) is usually a tailless IF protein with a stop codon located immediately after the final amino acid residue of helix 2B of the rod domain [15, 16]. The fish provide an exception to this rule. For example Zebra Fish, stickleback and both the Japanese and spotted-green puffer fish all have predicted tail domains [22], as observed for another fish, the trout [20]. The highly conserved TYRKLLEGE sequence at the end of the rod domain is characteristically conserved amongst the mammalian CP49 (BFSP2), with the sequence SYHALLDREE [22]. In contrast, CP49 (BFSP2) showed exceptional sequence divergence at the highly conserved helix initiation LNDR motif, resulting in the consistent substitution of arginine for cysteine [22]. The LNDR motif is usually critical for IF assembly [23, 24]. In other IF proteins, this very substitution is the genetic basis of many inherited human diseases, compromising IF assembly and its function [25]. There is a splice variant called CP49*ins*, containing an insertion of 49 amino acids within helix 1B of the rod domain [17, 26]. An extended rod domain is one of the hallmarks of ancestral IF proteins [25, 27], from which vertebrate cytoplasmic IFs are evolved. Indeed the IF proteins present in Squid and Octopus lenses bear such familiar hallmarks [28].

Both filensin (BFSP1) and CP49 (BFSP2) have a number of quite distinctive sequence features that distinguish them from the other cytoplasmic IFs. Interestingly, this sequence divergence continues between orthologues. Although one might expect a high degree of sequence homology (>85%) between orthologues from different species [29], this is neither the case for filensin (BFSP1) nor CP49 (BFSP2) [22]. For instance, the tail domain of filensin (BFSP1) is amongst the most variable regions between orthologues for all IF proteins [22]. The radical sequence changes observed for the filensin (BFSP1) and CP49 (BFSP2) sequences may reflect different functional requirements of the different lenses. For instance, eye accommodation differs between species requiring lens distortion in some species, but not in others [30]. It remains to be seen whether specific functions can be attributed to individual features in the sequences of these two proteins.

The assembly properties of the filensin (BFSP1) and CP49 (BFSP2)

With such distinctive sequence characteristics, it is perhaps not surprising that filensin (BFSP1) and CP49 (BFSP2) are also distinct in their assembly behaviour and the polymers that form, at least in vivo. In vitro assembly studies with purified filensin (BFSP1) and CP49 (BFSP2) have shown that each individual protein is incapable of forming conventional 10-nm filaments [31]. CP49 (BFSP2) can self-assemble into thin filament-like structure that associates laterally to form thicker bundles. Such thicker filamentous structures can also form from a variety of tail-truncated or tail-mutagenised IF proteins [32-36], suggesting that the tail domain is indeed important in controlling lateral associations between IFs [25] and highlighting the need for coassembly of CP49 (BFSP2) with filensin (BFSP1). On its own, filensin (BFSP1) can only form short, kinked fibrils [37, 38], which are reminiscent of those formed by NF-M and NF-H [39, 40]. When co-assembled in vitro, filensin (BFSP1) and CP49 (BFSP2) readily form heteropolymeric filaments with 10-nm morphology [31, 41].

This clearly demonstrates that filensin (BFSP1) and CP49 (BFSP2) require each other for filament assembly *in vitro*. The coassembly between filensin (BFSP1) and CP49 (BFSP2) is specific as neither filensin (BFSP1) nor CP49 (BFSP2) is able to co-assemble with vimentin in vitro, although recent studies suggested that filensin (BFSP1), or its proteolytic fragment(s), can interact with vimentin both in vitro [13] and *in vivo* [42]. In addition, CP49 (BFSP2) is unable to co-assemble with keratin, despite its relatedness to type I keratins [16]. The optimal molar stoichiometric of filensin (BFSP1) and CP49 (BFSP2) required for filament formation is reported to vary between 2:1 [31] and 3:1 [15], which is reminiscent of those formed by the neurofilament proteins.

Although filensin (BFSP1) and CP49 (BFSP2) coassemble into 10 nm IFs *in vitro*, they form a unique cytoskeletal structure called the beaded filament (Fig. 2A) in all vertebrate lenses as well as in squid [43]. The beaded filament has two distinct morphological features: a 6-8 nm filament backbone and a 12-15 nm bead that decorates the filaments at regular intervals. Some have reported that beaded filaments can be reconstituted from filensin (BFSP1) and CP49 (BFSP2) alone *in vitro* [38]. It is proposed that there is a core comprising four homotypic CP49 protofilaments that is then surrounded by the peripheral association of up to four heterotypic filensin/CP49 protofilaments [44]. It is thought that the long C-terminal tail projections of filensin (BFSP1) contribute to the regularly spaced beads [44].

The fact, however, that α-crystallins are a major component of the beaded filament fraction (Fig. 2B) and they associate very readily with filensin/CP49 filaments with similar bead spacing and dimensions of native beaded filaments [21] suggests that a complex with these protein chaperones is also very likely as previously proposed [45].

Functional studies of filensin (BFSP1) and CP49 (BFSP2)

Mouse knockout studies have shown that beaded filaments are essential for the optical properties of the lens by maintaining the three dimensional architecture of lens fibre cells [42]. Given the unusual sequence characteristics and their selective co-assembly properties, it has been suggested that filensin (BFSP1) and CP49 (BFSP2) play a functional role that is unique to the lens fibre cell biology. This hypothesis is tested by generating knockout animals with targeted deletion of the lens-specific IF gene *BFSP1* and *BFSP2* encoding filensin and CP49. Knockout of filensin (*BFSP1*) [46] and CP49 (*BFSP2*) [47, 48] each destabilised the other coassembly partner, resulting in the loss of beaded filaments. Although lenses from both knockouts show increased light scatter that progressively worsened with age, there appeared to be subtle differences between filensin (*BFSP1*) and CP49 (*BFSP2*) knockouts. The degree of light scatter in the filensin (*BFSP1*) knockout appeared to be greater, and at an earlier age [46], than CP49 (*BFSP2*) knockouts. In addition, the filensin heterozygotes also showed a slight increase in light scatter. The most important discovery, however, was the loss of optical function for the lenses of the *BFSP2* knockout mice [48]. Ultrastructural studies of the CP49 (*BFSP2*) lens revealed substantial changes in the fibre cell shape and plasma membrane organisation [48] as well as the loss of the beaded filament. Interestingly, the remaining filensin (BFSP1) in the CP49 (*BFSP2*) knockout appeared to associate with the vimentin filaments, dramatically altering the morphology of these filaments [42]. Table 1 summarises the phenotypes of filensin (*BFSP1*) and CP49 (*BFSP2*) knockout mice. These observations from *BFSP1* and *BFSP2* knockouts suggest that the filensin/CP49 filament network is required to maintain cell morphology and correct three-dimensional membrane architecture. Several studies have demonstrated the localisation of filensin (BFSP1) and CP49 (BFSP2) at the fibre cell membranes [4, 13, 49] and recent studies suggested that an aspect of this localisation is the association with the lens plasma membrane proteins, such as tropomodulin [50] and aquaporin 0 (AQP0) [51]. Of course such protein interactions have important functional implications, especially for such a major plasma membrane protein such as AQP0 and relates perhaps to the very tight association and specific binding of filensin (BFSP1) to lens plasma membranes [52].

A splice site mutation in BFSP2 mimics CP49 (BFSP2) knockouts

A natural mutation in *BFSP2* that mimics CP49 (*BFSP2*) knockouts has been reported in a wide range of mouse species, including CBA, 101, several 129 strains of mice [42, 53] and the FBV/N strain [54], which are popular for transgenesis. This mutation introduces a premature stop codon and will result in a severely truncated CP49 (BFSP2) protein if the mRNA is not destroyed by nonsense-mediated decay. Detailed analysis of the lens from 129 strains of mice has demonstrated that these mice lack CP49 (BFSP2). Filensin (BFSP1) levels were greatly reduced as seen in the knockout, but vimentin levels were unaffected. Ultrastructural analyses of the lens fibre cell cytoskeleton revealed the loss of beaded

filaments, which were replaced by poorly defined filament-like materials where vimentin was confirmed as one of the major components [42]. Indeed, this deletion mutation generates a natural CP49 (*BFSP2*) knockout because of the many features in common with the targeted deletion of *BFSP2* [47, 48].

Mutations in filensin (*BFSP1*) and CP49 (*BFSP2*) cause inherited cataract

There have now been two different mutations reported for CP49 (*BFSP2*), one in exon 3 (E233Δ; [55-57]) and the other in exon 4 (R287W; [58]). It is interesting to note that both residues are well conserved (Table 2), albeit the R287 residue is absolutely preserved in all animal sequences determined to date. Clearly a tryptophan substitution for arginine would be expected to have quite considerable impact given that the position of tryptophans is usually highly conserved in coiled coil proteins (eg [59]). The fact that the mutation introduces a second tryptophan just 8 residues away from the first (Table 2; W279) would be expected to compound the deleterious effects upon the coiled coil and the assembly of beaded filament. The mutation, however, appeared to be not fully penetrant as one carrier (IV:5; see [58]) certainly had good vision (corrected visual acuity 20:40 and 20:25) and no cataract. The age of cataract surgery for the other affected family members varied from 6-50 years and it was proposed that allelic heterogeneity might explain the variation.

The E233Δ mutation is the more interesting considering the fact that this has now occurred in three unrelated families across two continents [55-57]. Deletion of the residue E233 would be expected to completely alter the heptad repeat for CP49 (BFSP2) and be equally disruptive for beaded filament assembly. Given such a prediction, it is therefore perhaps a little surprising to find differences in the cataract phenotypes reported for the different families.

In the first family [55], there were phenotypic variations, but the light scattering properties of these cataracts meant that all affected individuals had surgery early in their life and the mutation appeared fully penetrant. The range of cataract phenotypes varied from nuclear to stellate or spoke-like cortical cataracts and also, sutural cataract, but this was the only eye phenotype reported. Subsequently there have been two Chinese family pedigrees reported. In one family from northern China, the E233Δ mutation caused a characteristic Y-shaped cataract at the lens sutures [56]. Loss in visual acuity required surgery for the affected individuals usually at an early age. Some phenotypic variability was noted, as one of the E233Δ carriers has not yet required surgery, but this is likely due in part to restricted location of the cataract.

In the other unrelated family from Southern China, the distinctive Y-shaped cataract at the lens sutures was again reported (Fig. 3), but this time it coincided with myopia [57]. The cataract usually started later in life after the first decade and progressed slowly resulting in the further development of punctuated cortical opacities that increased light scatter to the point that required surgery. Cataract developed more slowly in this family than the others that have been reported. One family member was found to have the E233Δ mutation (patient 31; [57]) but no loss in acuity despite presenting with a mild form of the Y-sutural cataract. Although three of the families examined here share the same E233 deletion mutation, there

are differences in terms of age of onset and severity and corresponding loss of visual acuity. It is clear that as with mutations in other IF proteins, some phenotypic variation will occur as seen in other IF-based diseases [60]. For instance, the same R94C mutation in keratin 17 causes typical Pachyonychia Congenita type-2 (PC-2) nail dystrophy in one family, but steatocystoma multiplex is the only phenotype in affected members of another family [61]. The I451M mutation in desmin provides another example, where this mutation was identified in two families with different phenotypes. Patients in the family reported by Li et al. [62] had cardiomyopathy with no sign of skeletal myopathy, whereas patients reported in another family [63] had progressive skeletal myopathy without cardiac involvement. These observations suggest that both the environment and epigenetic factors will likely contribute to the phenotypes observed and disease progression [24].

The first mutation in filensin (*BFSP1*) has now been reported and it is a recessive mutation caused by the deletion of exon 6 [64]. This introduces a premature stop codon after the addition of a novel hexapeptide sequence, but the introduction of this stop codon is expected to trigger nonsense-mediated decay of the mRNA, therefore effectively creating a filensin (*BFSP1*) knockout. In this family, homozygotes indeed presented with cortical cataracts in the first decade of life, but two heterozygote carriers (10, 16; [64]) presented with nuclear cataract at the age of 50. Whilst IF-related diseases caused by recessive mutation is considered to be rare, there have been a few cases of recessive keratin disease caused by homozygous mutations in keratin14 gene [65]. In one case, a homozygous deletion mutation led to a premature termination codon and complete loss of keratin14 expression presumably as a result of nonsense-mediated mRNA decay.

When these data are interpreted in conjunction with the mouse knockout studies, several observations emerge. The first is that filensin (BFSP1) and CP49 (BFSP2) are essential for lens function as described above. The second is that mutations in both genes can predispose individuals to cataract or other eye problems [58] such as age related (nuclear) cataract or even myopia. For instance, the E233 deletion mutation induced myopia in one family [57] concurs with the conclusion from one of the mouse CP49 (*BFSP2*) knockout studies [48]. Measurements of the back focal length of eyes from the CP49 (*BFSP2*) knockout animals indicated significant variation across the lens, which will likely distort the retinal image. The blurring of the image is not necessarily important; rather it is the relative spatial frequency composition of the image signal that reaches the retina that triggers myopia [66]. Cataract has more effect upon the shorter wavelength, higher energy light ([67] and discussion therein) and therefore mutations in either filensin (*BFSP1*) and CP49 (*BFSP2*) that interfere with the transmission of the visual signal through the lens by the deterioration of its optical properties, could potentially lead to myopia.

Filensin (*BFSP1*) and CP49 (*BFSP2*) mutations may also predispose individuals to nuclear cataract. For instance, two heterozygotic carriers of the filensin (*BFSP1*) exon 6 deletion presented with nuclear cataract later in life [64]. It is clearly worth following the other heterozygotes in this family to see if there is increased incidence of nuclear cataract in these individuals. Although there is a higher incidence (~3%) of nuclear cataract recorded for this part of India [68], it is still significant that two of the 19 family members presented with this type of cataract at a relatively early age [64]. By analogy, the lenses of the heterozygote

filensin (*BFSP1*) knockout mice had more light scatter than lenses from wild type litter mates [46], indicating a deterioration in lens function for these heterozygotes. A similar observation was not made for the heterozygote CP49 (*BFSP2*) knockout mice, suggesting that the influence of filensin (*BFSP1*) and CP49 (*BFSP2*) is not equivalent in the lens. What accounts for this difference is unclear, but could be related to the levels of unpartnered filensin (*BFSP1*) and CP49 (*BFSP2*). Studies on the keratin pair 8 and 18 demonstrate how disparity in protein levels of one IF protein (e.g. K8) in a partnership can cause pathology [69-71], and perhaps the same is true for filensin (*BFSP1*).

Previous studies using mouse models of cataractogenesis suggest the level of IF expression plays an important role in normal lens development and differentiation. For instance, over-expression of vimentin in the lens of transgenic mice results in abnormal fibre cell elongation and extensive fibre cell degeneration, eventually leading to lens cataract [72, 73]. The level of expressed vimentin is proportional to the severity of the lens opacity and the age of onset. These observations raise the possibility that vimentin could be a candidate gene for lens cataract. Indeed, it has been reported that expression of mutant vimentin in mice induces cataract formation [74]. Other IF proteins such as keratins [75], nestin [76] and synemin [77] are also present during lens development. They are, therefore, also potential candidate genes for new cases of inherited cataract.

Then another feature of both the human [55-58, 64] and the murine [78] studies is that the lens phenotypes for both point mutations, the deletion mutation and the targeted knockouts in mice, were progressive. This illustrates that ageing of the lens is a contributory factor in the phenotype attributed to the filensin (*BFSP1*) and CP49 (*BFSP2*) mutations and gene knockouts and is an important feature.

Inherited human cataract caused by IF-associated proteins

Cataract is not only caused by mutations in the lens IF proteins filensin (BFSP1) and CP49 (BFSP2), but it is also caused by mutations in genes encoding IF-associated proteins (Table 3). For instance, mutations in the small heat shock protein chaperones, αA-and αB-crystallin also cause inherited human cataract. As molecular chaperones, they can prevent stress-induced protein aggregation [79] and in the lens, both proteins are intimately associated with the IF cytoskeleton [80, 81] in the form of α-crystallin, a natural complex of both αA- and αB-crystallin. These chaperones help maintain the individuality of IFs by controlling filament-filament interactions [82] and assist in the formation of IF networks in cells [83]. The functional importance of αB-crystallin in the lens has been demonstrated by the discovery of a number of mutations causing inherited human cataract (Table 3). The R120G mutation alters αB-crystallin-IF interactions, promoting inter-filament interactions [83], but it should be noted that not all mutations in αB-crystallin cause cataract. Outside the lens, αB-crystallin mutations also cause human muscular disorders, including myofibrillary myopathies [84], desmin-related myopathies [85] and dilated cardiomyopathy [86]. It remains to be shown that αA- and αB-crystallin mutations cause human cataract by inducing beaded filament aggregation and a loss of function in the lens.

Conclusions and perspectives

The two lens-specific IF proteins filensin (BFSP1) and CP49 (BFSP2) have acquired a string of unique structural features that coincide with distinct assembly characteristics. Recent studies confirmed that filensin (BFSP1) and CP49 (BFSP2 are two key structural elements required for lens function (transparency, optical properties). The future elucidation of the precise functional roles that these proteins may play in the lens and the identification of interacting partners in the cytoplasm and at the plasma membrane are of fundamental importance to the understanding of lens fibre cell differentiation and the potential role that filensin (BFSP1) and CP49 (BFSP2) play in eye development and in cataractogenesis.

Acknowledgments

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Abbreviations

IF	intermediate filament
BFSP1 and 2	beaded filament structural protein 1 and 2
CP49	cytoskeletal protein 49
CP115	cytoskeletal protein 115

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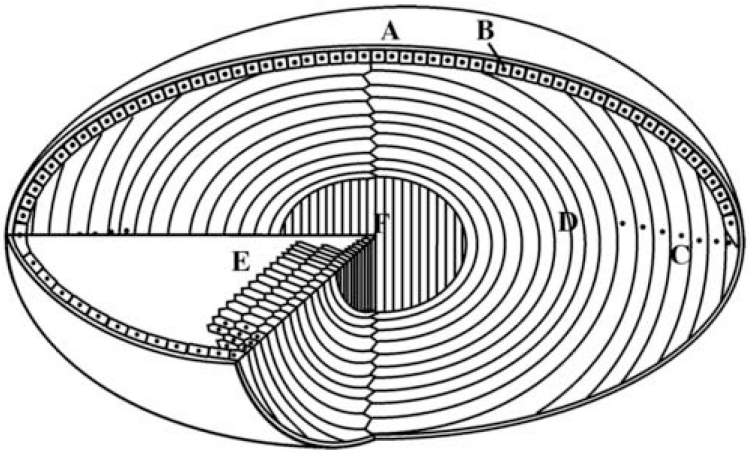

Figure 1.

Schematic view of the eye lens showing the main features and regions. The lens of the eye is enclosed by lens capsule (A) and the anterior surface of the lens is covered with a single layer of epithelial cells (B). The lens fibre cells are formed from epithelial cells at the lens equator (C) as part of their differentiation pathway. The epithelial elongate until their ends reach the two lens poles and are joined at the lens sutures. One of the striking features of the lens differentiation process at this stage is the removal of membrane-bound organelles (D) including nuclei, as indicated here as black dots. A cross section of the lens reveals the elongated fibre cells with characteristic hexagonal shape (E). The bulk of the lens thus consists of long, ribbon-like fibre cells arranged as concentric layer with the primary fibres cells at the centre of the lens (F).

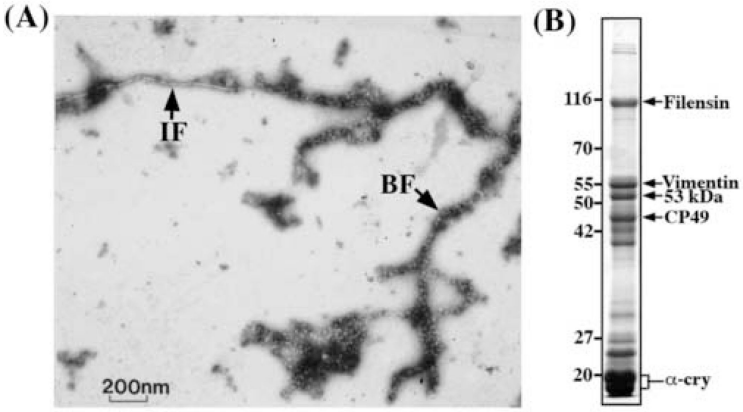

Figure 2.

(A) Transmission electron micrograph of native intermediate filament and beaded filament isolated from bovine lens. Samples were visualized by negative staining with uranyl acetate and a representative field is shown. The arrows denote intermediate filament (IF) with10-nm morphology and the typical beaded filament (BF) consisting of a filament backbone decorated with beads. Bar, 200 nm. (B) SDS-PAGE analysis of the cytoskeletal components prepare from the bovine lens cortex. The sample of final lens membrane pellet was analysed by 12% polyacrylamide gel and proteins were visualized by Coomassie blue staining. The proteins of interest include filensin (BFSP1), vimentin, 53 kDa filensin proteolytic fragment (53 kDa), CP49 (BFSP2) and α-crystallin are indicated by arrows. Molecular weight markers ($M_r \times 10^{-3}$) are shown and labeled on the left.

Figure 3.

Photograph showing the cataract found in a family with the E233Δ mutation. The photograph shows the typical Y appearance of the cataract that corresponds to the lens sutures – those places where the ends of individual lens fibre cells meet at the anterior and posterior parts of the lens (see [87] for an extensive review). This figure is reprinted from [57].

Table 1

Summary of the phenotypes of *BFSP1* (filensin) and *BFSP2* (CP49) knockouts

Knockouts	***BFSP1* (filensin)**	***BFSP2* (CP49)**
Lens phenotypes	No obvious change in lens morphology. Normal lens development and fibre cell differentiation	No obvious change in lens morphology. Substantial change in fibre cell membrane architecture
Optical property	Light scatter starts at 2 months, worsened with age	Loss of optical function that worsened with age
Cataract	No	No
Levels of assembly partner	Reduced	Reduced
Beaded filament	Lost	Lost, but vimentin filaments now have modified morphology
Heterozygous mice	Exhibited intermediate phenotype	Similar to WT litter mates

Table 2

Comparison of the amino acid sequences of residues 225-234 and 279-289 in the CP49 (BFSP2) from various species

ANIMAL	E233	R287
Mouse	EHQIESLK**E**E	WERDVEKN**R**AE
Rat	EHQIESLK**E**E	WERDVEKN**R**AE
Rabbit		WERDVEKN**R**VE
Cow	ESQIESLK**E**E	WERDVEKN**R**LQ
Elephant		WERDVEKS**R**VE
Dog	ESQIESLK**E**E	WERDVEKN**R**AE
Man	ESQIESLK**E**E	WERDVEKN**R**VE
Chimp	ESQIESLK**E**E	WERNVEKN**R**VE
Macaque*	ESQIESLK**E**E	WEEEEEKL**R**EE
Hedgehog	ESQIEGLK**E**E	WERDVEKK**R**ME
Opossum	ESQIESMK**E**E	WEKDIEKN**R**AE
Platypus	ESQIESMK**E**E	
Chick	ESQIESMK**E**E	WERDIEKN**R**AE
Puffer-SpottedGreen	EEHMEDLRAE	WEKVSEKN**R**AE
Puffer-Japanese	EEQMEDLRAE	WEKVTEKN**R**AE
Trout	ESQMEDLRAE	WERVIEKN**R**AE
Zebrafish*	ESMKTENV**E**Q	WERVVEKN**R**AE
Stickleback	EEQMENMRAD	WEKVMERN**R**AE
Medaka	EEQMEMMRQE	WEKVTEKN**R**VE

The identified mutations (E233 and R287) are highlighted. Sequences were extracted from the ENSEMBL database release 42 using the human gene *BFSP2* (ENSG00000170819) for reference and using the orthologue prediction section to trace sequences in the other animals. The asterisk indicates sequences that have been deduced from ESTs and database mining to address obvious database errors. For example the entry ENSBTAP00000024638 for the bovine Bfsp2 contains an error in exon assignment and translation when compared to the NCBI entry NM_174248 which affects the sequence around E233.

Table 3

Inherited human cataract caused by mutations in IF-associated proteins

Disease	Mutation	Ref.
Autosomal dominant cataract	αA-Cry (R49C)	[88]
Congenital cataract	αA-Cry (G98R)	[89]
Autosomal dominant congenital cataract	αA-Cry (R116C)	[90]
Autosomal dominant posterior polar cataract	αB-Cry (P20S)	[91]
Cataract and desmin-related myopathy	αB-Cry (R120G)	[92]
Autosomal dominant congenital lamellar cataract	αB-Cry (D140N)	[93]
Dominant congenital posterior polar cataract	αB-Cry (450ΔA)	[94]
Dominant congenital cataract	Aquaporin 0 (missense)	[95]
Autosomal dominant congenital cataract	Aquaporin 0 (deletion)	[96]