Mutations in spliceosomal proteins and retina degeneration

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ABSTRACT
A majority of human genes contain non-coding intervening sequences – introns that must be precisely excised from the pre-mRNA molecule. This event requires the coordinated action of five major small nuclear ribonucleoprotein particles (snRNPs) along with additional non-snRNP splicing proteins. Introns must be removed with nucleotidial precision, since even a single nucleotide mistake would result in a reading frame shift and production of a non-functional protein. Numerous human inherited diseases are caused by mutations that affect splicing, including mutations in proteins which are directly involved in splicing catalysis. One of the most common hereditary diseases associated with mutations in core splicing proteins is retinitis pigmentosa (RP). So far, mutations in more than 70 genes have been connected to RP. While the majority of mutated genes are expressed specifically in the retina, eight target genes encode for ubiquitous core snRNP proteins (Prpf3, Prpf4, Prpf6, Prpf8, Prpf31, and SNRNP200/Br2) and splicing factors (RP9 and DHX38). Why mutations in spliceosomal proteins, which are essential in nearly every cell in the body, causes a disease that displays such a tissue-specific phenotype is currently a mystery. In this review, we recapitulate snRNP functions, summarize the missense mutations which are found in spliceosomal proteins as well as their impact on protein functions and discuss specific models which may explain why the retina is sensitive to these mutations.

Introduction
A vast majority of human genes contain non-coding intervening sequences – introns - that are removed from mRNA molecules during splicing. Intron excision and exon joining requires two transesterification reactions which are catalyzed by a large ribonucleoprotein complex called the spliceosome. The spliceosome dynamically changes its composition during splicing with the overall number of involved proteins reaching 170. In addition, the spliceosome contains five small nuclear RNAs (snRNAs), which together with ~65 proteins, enter splicing as preassembled small nuclear ribonucleoprotein particles (snRNPs) (reviewed in1–6).

Various human inherited diseases are caused by mutations that directly affect the accuracy and efficiency of pre-mRNA splicing.7,8 One of the most interesting genetic mysteries is represented by inheritable retinitis pigmentosa (RP), which is caused by the mutation of more than 70 genes (sph.uth.edu/retnet). A majority of target genes are expressed specifically in the retina and are involved in different aspects of photoreceptor function (light detection, signal transduction, membrane components or structural elements of retina cells).3 Surprisingly, mutations found in six small nuclear ribonucleoprotein particle (snRNP)-specific proteins (Table 1) and two non-snRNP splicing factors have been linked to RP as well. These ubiquitously expressed proteins are essential for almost every cell in the body and it remains enigmatic why mutations in these genes are associated with such a tissue specific phenotype.

RP is the most common type of inherited retinal degeneration characterized by the progressive loss of photoreceptor cells. It occurs in 1 in 3,000–5,000 individuals, without any apparent link to ethnic or racial background.9 Pathological changes include atrophy of the retinal pigment epithelium (RPE), retina vessel attenuation, constricted visual fields, waxy pallor of the optic disc and the degeneration of photoreceptor cone and rod cells. Symptoms first appear as night blindness progressing to loss of peripheral vision, eventually leading to total blindness. However, the age of onset and the rate of progression varies among individuals. These two characteristics are likely dependent on genetic background.5,10 RP can be transmitted in all three forms of mendelian inheritance: autosomal dominant (adRP), autosomal recessive (arRP) and X-linked (xLRP). 30%–40% cases account for adRP of which a quarter of these are caused by mutations in the rhodopsin gene. arRP represents about half of RP cases and ~10% of RP cases are X-linked.11

Small nuclear ribonucleoprotein particles
Spliceosomal snRNPs are complex particles consisting of small nuclear RNA (snRNA), a heptamerlic ring of Sm or Like-Sm (LSm) proteins and 1–13 proteins that are specific for each snRNP. Five major and four minor snRNPs have been described and named according to their snRNA composition. The major snRNPs are U1, U2, U4, U5 and U6 and the minor snRNPs are U11, U12, U4atac and U6atac. The U5 snRNP is common to both major and minor splicing pathways.

The most accepted view of spliceosome formation is a stepwise assembly model, which has been supported by numerous
in vitro and in vivo studies (e.g.7,8; reviewed in2). First, U1 snRNP interacts with the 5′ splice site via RNA-RNA base pairing of the U1 snRNA with the sequence at the 5′ splice site. This interaction is strengthened by the U1-C protein.9 The sequence around the branch point is initially recognized by proteins SF1 and U2AF35/65,10 which are subsequently replaced by U2 snRNP. Subsequently, the U2 snRNA forms a duplex with the intron sequence surrounding the adenine at the branch point.11

After U1 and U2 snRNPs define intron boundaries, U4/U6•U5 tri-snRNP joins the spliceosome. The U4-U6 helix is disrupted by the DExD/H box helicase SNRNP200 (also referred to as hBrr2).12,14 While U4 snRNA together with U4/U6-specific proteins (including proteins mutated in RP Prpf3, Prpf4 and Prpf8 which are described in detail below) leave the spliceosome, U6 snRNA replaces U1 snRNA at the 5′ splice site resulting in a new interaction with U2 snRNA. U1 snRNP is released and a catalytically competent spliceosome is formed. Several studies have suggested that the active splice site is formed by a dynamic snRNA-pre-mRNA network.15-18 The crucial role of snRNAs, namely the U2, U5 and U6 snRNAs, in the splicing reaction was recently confirmed in Saccharomyces pombe where the spliceosome structure was elucidated.19,20 snRNP proteins, mainly Prpf8, hold pre-mRNA in the correct orientation and provide structural support for snRNAs in the active site. During and after splicing, the spliceosome undergoes many structural rearrangements that are mediated by several RNA helicases including SNRNP200 and DHX38 (hPrp16) (21, reviewed in1). Post-splicing snRNPs bound to the intron lariat are removed by the Prp43/Ntr1/Ntr2 complex. The lariat is then rapidly degraded and snRNPs are recycled for the next splicing reaction.22

In addition to splicing function, snRNPs, namely U1 snRNP, have been implicated in other nuclear processes, namely RNA polymerase II transcription and U1 snRNP associates with RNA polymerase II (Pol II).23 The protein FUS, which interacts with both U1 snRNP and Pol II, was suggested to mediate Pol II-U1 association.24 FUS mutations have been linked to the neurodegenerative disease, amyotrophic lateral sclerosis (ALS).25 Interestingly, U1 snRNP-specific proteins also interact with SMN protein, which is linked to another motor neuron degenerative disease with early onset - spinal muscular atrophy.26,27 It has been observed that depletion of U1 snRNP proteins leads to reduced growth of motor neurons.26 This suggests that U1 snRNP might be a common link between diseases that affect motor neurons.

**Mutations in splicing factors linked with retinitis pigmentosa**

In this chapter, we summarize the current information regarding exonic missense mutations in six snRNP-specific and two non-snRNP proteins that are associated with RP. We have mostly omitted discussion related to deletions or mutations that affect splicing, which often lead to a reading frame shift, resulting in the introduction of a premature stop codon and subsequent destabilization of mRNA or protein. So far, RP linked mutations were identified only in U4/U6•U5 tri-snRNP-specific proteins. No mutations associated with RP have been detected in U1 or U2 snRNP-specific proteins (Table 1 and 2).

**Prpf3**

Prpf3 is a 90 kD protein, which forms a stable trimeric complex with Prpf4 and PPIH. The complex binds the U4/U6 snRNA duplex primarily via the interaction of Prpf3 with U6 and U4/U6 RNA.28,29 Prpf3 further stabilizes the U4/U6•U5 tri-snRNP by interacting with Prpf6 and Sart1.29,30 The C-terminus of Prpf3 is required for binding to RP9 (PAP-1), a protein also implicated in adRP.31

Two different missense mutations were discovered in two adjacent codons in exon 11 of the gene **Prpf3**, causing amino acid substitutions Thr494Met and Pro493Ser.32 Finally, a third mutation in the same exon, which was identified in a Spanish family, was found to be responsible for the substitution at Ala489Asp.33 Thr494Met is the most frequently observed substitution in Prpf3 while Ala489Asp occurs rather sporadically.
All three mutations are clustered in the C-terminal conserved region, which is important for protein-protein interactions. So far, only the Thr494Met mutation has been extensively studied. Mutation Thr494Met causes aggregation of the mutated protein when expressed in 661 W cells, which are used as in vitro models of photoreceptor cells. Nuclear aggregates were not observed when the Prpf3<sup>Thr494Met</sup> protein was expressed in HEK. Thr494 overlaps with a potential recognition motif for phosphorylation by casein kinase II (CKII). Gonzales-Santos and colleagues showed that Thr494Met substitution reduced Prpf3 phosphorylation and impaired the association with Prpf4 and U4/U6 snRNP. Consistently, patients who express mutated Prpf3 exhibited altered levels of snRNPs, delayed spliceosome assembly and inefficient splicing. The function of Prpf3 has also yielded interesting results in mice models. It was shown that Prpf3<sup>Thr494Met</sup> heterozygotic mice developed normally exhibiting compensatory expression from the Prpf3 wild-type allele. However, pathological changes in the retinal pigment epithelium (RPE) of Prpf3<sup>Thr494Met</sup> knock-in mice were detected in two year old mice. Further research has revealed additional defects in RPE phagocytic function in Prpf3<sup>Thr494Met</sup>/Thr494Met mice, which points to RPE as a primary target tissue and photoreceptor death as a consequence of RPE dysfunction. However, two year mice are at the end of their life and thus this results should be considered with caution.

**Prpf4**

Prpf4 is a 60 kD protein that complexes with Prpf3 and PPIH and is part of both U4/U6 and U4/U6•U5 snRNPs. In 2011, Linder and his colleagues suggested that Prpf4 might be involved in RP because Prpf4 reduction caused the same eye phenotype as reduced expression of Prpf3, a protein linked to RP (see below). Indeed, two amino acid substitutions (Arg192His and Pro315Leu) in the **PRPF4** gene have been recently connected with RP. In addition, a third mutation that causes the substitution of Pro187Ala was found in a patient with adRP, but the connection of this substitution to the disease phenotype was not fully established. Cells expressing the mutated Prpf4<sup>Pro315Leu</sup> exhibited altered levels of splicing factors as well as changes in the nuclear morphology of the splicing factor compartment. Detailed molecular analysis showed that a Arg192His substitution disrupts the association of Prpf4<sup>Arg192His</sup> with Prpf3 and its incorporation into snRNPs. The reduced expression of Prpf4 protein in zebrafish led to morphological changes and degeneration of photoreceptor cells. Additionally, the expression of Prpf4<sup>Pro315Leu</sup> and Prpf4<sup>Arg192His</sup> proteins in zebrafish were shown to be linked to embryonic and retinal defects.

**Prpf6**

Prpf6 is a 102 kD protein U5 snRNP-associated protein that is essential for the interaction between U5 and U4/U6 particles and the formation of U4/U6•U5 snRNPs. Only one mutation in **PRPF6** gene has been identified thus far, c.2185C>T transition in the TPR domain resulting in Arg729Trp substitution. The mutated Prpf6<sup>Arg729Trp</sup> has been shown to accumulate in Cajal bodies. Because defective snRNPs accumulate in these nuclear structures, this finding suggests that Arg729Trp substitution inhibits snRNP assembly. The Arg729 is in a close proximity to Prpf4 suggesting that it is involved in formation and stabilization of the tri-snRNP. In addition, inefficiently spliced pre-mRNAs were detected in patient cells expressing Prpf6<sup>Arg729Trp</sup>. Interestingly, a missense mutation, c.1430A>G, found within the **PRPF6** gene co-segregated with progressive neurodegenerative genetic Kufs disease. It was observed that patients who carried this missense mutation suffered visual impairment. However, additional studies are needed to confirm the role of this mutation in either Kufs disease or the visual defect.

**Prpf8**

At 220kD, Prpf8 is the largest protein found in the spliceosome. Prpf8 is a highly conserved protein that lies in the center of the spliceosome where interacts with the U5 snRNA as well as the 5’ and 3’ splice sites of pre-mRNA. Eleven missense mutations causing amino acid substitutions were found in the **PRPF8** gene and have been linked to adRP (Table 2). Currently, all mutations identified cluster in the C-terminal Jab1/MPN domain, which interacts with SNRNP200 and regulates its helicase activity. The Jab1/MPN domain has apparently two opposing functions in SNRNP200 regulation. While the entire Jab1/MPN domain stimulates SNRNP200 helicase activity, the C-terminal tail is inserted into the active site of the SNRNP200 helicase and sterically inhibits its activity. Consistently, RP mutations that shorten the C-terminus increase the stimulatory effect, likely by removing the inhibitory tail. It has been also described that the mutation reducing Prpf8-SNRNP200 interaction has a negative effect on helicase stimulation. Coincidently, SNRNP200 is also linked to RP and mutations within SNRNP200 are known to reduce its helicase activity.

The effect of **PRPF8** mutations in cell metabolism was studied mostly in yeast providing similar, but not fully consistent, results exhibiting different growth defects which are likely dependent on the genetic background of the studied yeast strain. However, experiments in yeast have pointed to an additional defect caused by RP mutations - inhibition of U5 snRNP assembly. Several labs have reported that yeast Prp8p mutated variants mimicking human Prpf8 RP mutations interacted less efficiently with U5 proteins yBrr2p and ySnu114p, which resulted in splicing defects. Similarly, inefficient spliceosome assembly and reduced splicing were detected in cells derived from RP patients carrying **PRPF8** mutations.

Currently, it is unclear how mutated Prpf8s induce the RP phenotype. One possibility is via misregulation of SNRNP200 resulting in changes in splicing. Alternatively, Prpf8 proteins containing exonic substitutions may inhibit the formation of snRNPs and thus reduce the effective concentration of splicing competent snRNPs. Recently, two studies observed gene specific splicing defects after Prpf8 knockdown suggesting that the reduction of Prpf8 concentration can target only a subset of susceptible genes. Interestingly, several somatic **PRPF8** missense mutations were identified in patients with myeloid syndromes such as acute myeloid leukemia. Although, the presence of **PRPF8** mutations in patients with acute myeloid leukemia was associated with a poor disease prognosis, their
The human malignant phenotype is currently unknown. Impact on the factors linked to RP.

Table 2. List of missense mutations and amino acid substitutions of spliceosomal factors linked to RP.

| Gene/Exon No. | Nucleotide substitution | Amino acid substitution | Reference |
|---------------|-------------------------|-------------------------|-----------|
| Prpf3         | c.1466C>A               | Ala489Asp               | 33        |
| 11            | c.1478C>T               | Pro493Ser               | 32        |
| 11            | c.1482C>T               | Thr494Met               | 32,94     |
| Prpf4         | c.556C>G                | Pro187Ala*              | 43        |
| 5             | c.675G>A                | Arg192His               | 42        |
| 6             | c.944C>T                | Pro315Leu               | 41        |
| Prpf6         | c.2185C>T               | Arg729Trp               | 46        |
| Prpf8         | c.6353C>T               | Ser2118Phes              | 99        |
| 38            | c.6901C>A               | Pro2301Thr              | 100       |
| 42            | c.6901C>T               | Pro2301Ser              | 101       |
| 42            | c.6912C>G               | Phe2304Leu              | 100       |
| 42            | c.6926A>C               | His2309Pro              | 100       |
| 42            | c.6926A>G               | His2309Arg              | 100       |
| 42            | c.6926G>A               | Arg2310Lys              | 100       |
| 42            | c.6928A>G               | Arg2310Gly              | 99        |
| 42            | c.6930G>C               | Arg2310Ser              | 100       |
| 42            | c.6942A>C               | Phe2314Leu              | 100       |
| 42            | c.7001T>A               | Tyr2334Asn              | 102       |
| 42            | c.7006T>C               | Term2336Arg             | 98        |
| Prpf31        | c.319C>G                | Leu107Val               | 66        |
| 3             | c.413C>A                | Thr138Lys               | 103       |
| 4             | c.581C>A                | Ala194Glu               | 60        |
| 6             | c.646C>G                | Ala216Pro               | 60        |
| 6             | c.669G>A                | Gly224Arg               | 104       |
| 8             | c.871G>T                | Asp291Pro               | 86        |
| 8             | c.895T>C                | Cys299Arg               | 86        |
| SNRNP200/Brr2 | c.2041C>T               | Arg681Cys               | 81        |
| 16            | c.2042G>A               | Arg681His               | 81,105    |
| 16            | c.2504G>T               | Val683Leu               | 81        |
| 16            | c.2066A>G               | Tyr689Cys               | 81        |
| 20            | c.2653G>A               | Gin885Glu               | 80        |
| 25            | c.3260C>T               | Ser1087Leu              | 78,81     |
| 25            | c.3269G>T               | Arg1090Leu              | 106       |
| DHX38/Prpf16  | c.995G>A                | Gly332Asp               | 91        |
| RP9/PAP1      | c.509A>G                | Asp170Gly               | 83        |

Notes.
* Link to RP phenotype is not fully established
** Extension of the protein by 41aa
*** This mutation causes partial skipping of exon 4 resulting in a frameshift

Impact on the final function of the protein as well as on the malignant phenotype is currently unknown.

**Prpf31**

The human **PRPF31** gene is comprised of 14 exons and encodes a highly conserved 61 kDa protein of 499 amino acids.45 Prpf31 is an essential protein that is involved in the assembly and stability of the U4/U6•U5 tri-snRNP. Prpf31 is part of the U4 snRNP, which binds the U5-specific protein Prpf6, creating a bond which is essential for the formation of the splicing competent U4/U6•U5 tri-snRNP.30,45,59

Mutations in **PRPF31** were first described 15 year ago by Vithana et al.60 To date, ~60 mutations have been reported according to [http://www.hgmd.cf.ac.uk](http://www.hgmd.cf.ac.uk). Most mutations in **PRPF31** are deletions, frameshifts or mutations that inhibit splicing and thus include introns that contain many premature stop codons. All of these types of mutations destabilize Prpf31 mRNA, which results in reduced concentration of functional Prpf31, which has been suggested to be a major cause of RP in patients carrying **PRPF31** mutations.61,66 The correlation between reduced Prpf31 expression and eye malfunction was further confirmed in mouse, zebrafish and fruit fly model organisms.39,40,67 However, several individuals carrying mutated **PRPF31** did not present RP symptoms. It was shown that Prpf31 insufficiency can be compensated by higher expression from a wild type allele. Several genomic loci that enhance Prpf31 expression and prevent disease development have been identified.61,66,68,69

In addition to mutations reducing the level of Prpf31 mRNA, several missense mutations, which segregate with RP, have been described. Two of these substitutions, Ala194Glu and Ala216Pro, found in the NOP domain, which has been identified in several RNP proteins. Both mutated variants exhibit reduced import to the cell nucleus.70,71 It was further determined that Prpf31Ala216Pro was not properly incorporated into U4/U6 snRNP but surprisingly, Prpf31Ala216Pro interacted with U5-specific Prpf6 both in vitro and in vivo.72,73 These results suggest a cytotoxic effect of the Ala216Pro substitution via the blocking of Prpf6 and thus preventing U4/U6 snRNP interaction with U5 snRNP. Interestingly, this negative effect of Prpf31Ala216Pro can be reverted by higher Prpf6 expression.72 Consistently, the Prpf31 mutation reduces the concentration of active snRNPs as well as the splicing efficiency of reporter and endogenous genes.36,72,73

**SNRNP200/Brr2**

SNRNP200 is a large nuclear protein of 200 kDa, which interacts extensively with U5-specific proteins EFTUD2 (hSnu114) and Prpf8. SNRNP200 is a DExD/H box RNA helicase composed of two helicase modules, one active and the other possessing a regulatory function.74,75 SNRNP200 unwinds U4/U6 snRNAs during spliceosome activation and the helicase activity is regulated by the C-terminus of Prpf8 and GTPase activity of EFTUD2 proteins.52,53,76,77 To date, six mutations have been identified in SNRNP200 (Table 2). Two mutations (Ser1087Leu and Arg1090Leu) are in the Sec63-like domain of the enzymatically active N-terminal helicase module. Both of these mutations reduce unwinding activity and promote usage of cryptic splice sites, which indicate an influence on splicing fidelity.78,79 Interestingly, in contrast to mutations in other snRNP proteins, these two mutations do not affect snRNP formation and are normally incorporated into snRNPs.79 Five mutations (Arg681Cys, Arg681His, Val683-Leu, Tyr689Cys and Gin885Glu) were recently found in the Ski2-like helicase domain of the N-terminal helicase module.40,81 These data together with mutations found in the Prpf8 domain, which regulates SNRNP200 function, suggests that malfunction or ill-timing of SNRNP200 has a deleterious effect on splicing resulting in cell death.
RP9/PAP-1

RP9 (also called PAP-1) is a 221 amino acid protein originally identified as a target and an interacting partner of Pim-1 kinase.\textsuperscript{82} Later, two amino acid substitutions within RP9 (His137Leu and Asp170Gly) were associated with adRP.\textsuperscript{83} RP9 protein localizes to the nuclear splicing factor compartment, which contains many splicing factors. In addition, RP9 interacts with Prpf3, a U4/U6 snRNP specific protein.\textsuperscript{31} The authors who described the RP9 interaction with Prpf3 also observed an association of RP9 with tri-snRNPs, however, other studies analyzing the composition of snRNPs did not detected RP9 protein in di- or tri-snRNPs.\textsuperscript{84} RP9 exhibits splicing activity both in vitro and in vivo but the exact role of RP9 in splicing remains to be clarified. It should be mentioned that only the RP9\textsuperscript{Asp170Gly} mutated variant exhibited splicing defects.\textsuperscript{85} A pseudogene of the RP9 gene was found at the same chromosomal locus. Two different studies reported that the RP9 pseudogene carries mutations that cause either His137Leu\textsuperscript{86} or Asp170Gly substitutions.\textsuperscript{87} These findings together with the unclear function of RP9 during splicing, presents the question of whether mutations in the RP9 gene are causative in adRP.

DHX38

DHX38 is a 140 kDa DEAH-box helicase which acts during splicing. Its function was extensively studied in yeast where the DHX38 homolog, Prp16p, interacts with the Nineteen complex and Brr2p (the yeast homolog of SNRNP200).\textsuperscript{88,89} Recent data suggest that Prp16p is important for Nineteen complex mediated remodeling of U2-U6 snRNA helix during splicing.\textsuperscript{90} In 2014, Ajmal et al. found that a homozygotic single nucleotide mutation, which causes Gly332Asp substitution, correlated with early onset RP.\textsuperscript{91} Thus, this is the first example of a recessive mutation in a splicing factor linked to RP.

Conclusions

The list of RP mutations in splicing proteins and their diverse effects on protein functions does not provide a clear clue of the molecular mechanisms underlying the RP phenotype. One mutation depicts only a mild splicing phenotype while another one significantly limits protein function. Nevertheless, splicing defects are most often mentioned when explaining adRP which is caused by mutations in genes that code for splicing proteins. Retina cells express unusually high amounts of spliceosome components, which indicates a high demand for splicing.\textsuperscript{36,92} Moreover, inefficient splicing and alterations in alternative splicing have been reported in cell culture experiments, animal models and blood cells derived from RP patients. However, it should be mentioned that general splicing defects do not always lead to retina degeneration because mutation of splicing factors FUS and SMN cause motor neuron degeneration while the photoreceptors seem to be not affected. The explanation of these differences could lie in recent studies that analyzed splicing after depletion of various splicing factors. First, Pleiss et al. showed in yeast that the depletion of core splicing proteins resulted in transcript specific splicing defects.\textsuperscript{93} In human cell culture, the knockdown of different core splicing factors exhibited a gene-specific alternative splicing phenotype. In addition, different tri-snRNP-specific proteins affected the same set of genes.\textsuperscript{57} Finally, the reduction of Prpf8 resulted in gene-specific splicing changes and affected mainly genes with weak 5′ splice site.\textsuperscript{56} This differential sensitivity of various genes to a reduced concentration of core splicing factors could explain the tissue specific phenotype of mutations in generic splicing factors. Mutations in tri-snRNP proteins affect all splicing events but some retina specific transcripts are overly sensitive to reduced levels of functional tri-snRNP. Potential targets could include genes, which are involved in retinal metabolism or those genes that protect against light induced oxidative damage, to which
retina cells are quite susceptible. However, retina-specific mis-spliced variants have not yet been identified and further research is necessary to compare splicing variants in wild-type retina and retina expressing mutated proteins. It would be also interesting to see whether splicing defects are in major U2 introns or in U12 introns as was suggested for SMA.

Another interesting question is why many patients carrying splicing mutations develop RP in the second or third decade of their life. A similar later onset has been documented for patients with ALS as well. Two potential splicing factors FUS and TDP-43 were found mutated in several ALS patients and in both cases mutations stimulated protein aggregation. Recently, it was shown that FUS protein aggregation increases over time and is enhanced by ALS mutations. Many RP mutations in splicing factors abrogate their incorporation into mature snRNPs, likely by inhibiting proper protein folding. Is it thus possible that protein aging, as suggested in several neurodegenerative disorders and laminopathies, is responsible for RP phenotypes? Similarly to prions, a mutation in a splicing factor induces protein-folding defects that slowly propagate in a healthy protein population and inactivates it. Indeed, large aggregates and amorphous deposits have been observed in photoreceptors and RPE expressing mutated proteins. It should be also noted that photoreceptor cells do not regenerate and thus any protein defect or stress will accumulate over time possibly resulting in an increased probability of cell death. An alternative hypothesis is based on the unfolded protein response, which detects mis-folded proteins and alter cellular metabolism. Constant production of mis-folded snRNP proteins creates long-lasting stress that together with photooxidative damage, retina cells are prone to, trigger apoptosis. Consistently, it was shown recently that mutation in the RHO gene promotes retinal degeneration in rats likely via persistent activation of unfolded protein response and induced changes in calcium homeostasis.

Finally, findings that mice expressing RP mutants exhibit primary defects in the phagocytic function of RPE as opposed to photoreceptors indicate that we are still far from a complete understanding of molecular principles underlying RP. Hence, further research is necessary to elucidate the molecular cause of the disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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