PŘÍRODOVĚDECKÁ FAKULTA UNIVERZITY KARLOVY V PRAZE
KATEDRA BUNĚČNÉ BIOLOGIE

Mutations of Splicing Factors in Hereditary Eye Disease – Retinitis Pigmentosa

Petr Těšina

BAKALÁŘSKÁ PRÁCE

Vedoucí bakalářské práce: Mgr. David Staněk, Ph.D.
Ústav molekulární genetiky Akademie věd České republiky, v. v .i.

2008

Table of contents

Abstrakt

Retinitis pigmentosa je dědičné onemocnění oka způsobující progresivní odumírání fotoreceptorů, čímž dochází k nevratnému poškozování zraku až k úplnému oslepnutí.

Přestože toto onemocnění postihuje jen přibližně jednoho z každých čtyř tisíc lidí, jde o jednu z nejčastějších příčin poškození zraku nebo slepoty.

Z řady genů zodpovědných za vznik tohoto onemocnění vyčnívají čtyři, které nejsou exprimovány specificky v buňkách fotoreceptorů. Jsou to geny kódující sestřihové faktory nezbytné pro sestavení a funkci sestřihového komplexu – spliceosomu.

Jelikož kódující sekvence lidských pre-mRNA (exony) jsou zpravidla přerušeny různým počtem sekvencí nekódujících (introny), je pre-mRNA sestřih, tedy proces odstranění intronů a spojení exonů, nezbytným krokem pro vznik funkční mRNA kódující žádaný produkt.

Je proto překvapivé, že mutantní formy těchto sestřihových faktorů účastnících se procesu nezbytného pro přežití kterékoliv lidské buňky, způsobují onemocnění postihující specificky fotoreceptory.

Mechanismy tohoto působení zůstávají nejasné, navzdory snaze o funkční charakterizaci jednotlivých mutací.

Klíčová slova

retinitis pigmentosa, dědičná choroba, retinopatie, pre-mRNA sestřih, střihové faktory, hPrp31, PRPF31, hPrp3, PRPF3

Abstract

Retinitis pigmentosa is a hereditary eye disease causing progressive loss of photoreceptor cells, which leads to an irreversible sight handicap and eventually complete blindness.
Although the prevalence of the disease is only about one of every four thousand, it is still a major cause of visual handicap or blindness.
There are four genes accounting for retinitis pigmentosa that encode splicing factors necessary for spliceosomal assembly and function. Unlike any of the other known genes associated with this disease, these are all expressed ubiquitously throughout the human body.
Typically, human pre-mRNAs are abundant in intervening noncoding sequences (introns) that interrupt the coding sequence elements (exons). Therefore splicing (the process through which introns are excised and exons joined) represents a fundamental step in pre-mRNA maturation.
Intriguingly, the mutant forms of these splicing factors necessary for survival of any human cell cause cell-type specific disease affecting only photoreceptor cones and rods.
Molecular mechanisms underlying this cell-type specific effect remain elusive, despite the data gained in the studies of mutations in these splicing factors.

Keywords

retinitis pigmentosa, hereditary disease, retinopathy, pre-mRNA splicing, splicing factors, hPrp31, PRPF31, hPrp3, PRPF3

Preamble

In this paper I would like to summarize available data concerning mutations of splicing factors associated with retinitis pigmentosa.

In order to get on with this theme, which is a current research front, I will need to gain some deeper insight into the basis at first. By the basis I mean the disease itself and the process of pre-mRNA splicing in humans.

Therefore, I will divide this paper into three chapters. Each of them will represent a short review of the particular themes.

Whereas the first two chapters should be rather general, in the third one I would like to compile the functions of respective splicing factors, proposed models of cell-specific pathogenicity mechanisms of the mutations and results of their functional characterization where available, which may either support or contradict some of the proposed models.

In the end I would like to draw a conclusion expressing my own opinion and consider possible implications for my future work.

1. Retinitis pigmentosa

1.1 Definition and description

Retinitis pigmentosa (RP) refers to a group of inherited disorders that lead to blindness due to abnormalities and progressive loss of photoreceptor rods and cones cells. It is a major cause of visual handicap or blindness accounting for 25% of patients [1].

The cones are responsible for sharp central vision and colour vision. They are primarily located in a small area of the retina called the fovea and gradually become more sparse towards the periphery of the retina. The rods are concentrated at the periphery of the retina and are responsible for peripheral and night (scotopic) vision.

Another layer of the retina - the retinal pigmented epithelium (RPE), may also be affected. The retinal pigment epithelium is the pigmented cell layer outside the neurosensory retina that nourishes the photoreceptors by maintaining homeostasis of environment, supplying nutrients and phagocytosing outer segments of photoreceptor cells.

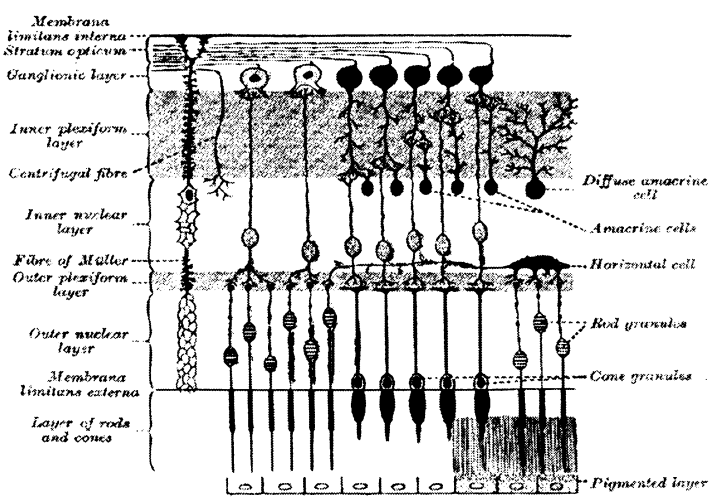


Figure 1: Schematic section of retina - two lowermost cell layers are affected by RP
Adopted from Gray's Anatomy

Retinitis pigmentosa is a rather uncommon disease with prevalence of approximately 1 out of every 4000 [2] people in the United States and Europe, which would mean about 1.5 million affected individuals worldwide (this is just estimation due to the lack of data from other parts of the world).

1.2 Symptoms and diagnosis

Retinitis pigmentosa is a highly variable disorder. However, most of the cases fall into the typical course of RP, which is a primary degeneration of photoreceptor rods followed by secondary degeneration of cones (rod-cone dystrophy). However, the course may be right opposite in some atypical forms affecting the cones first (cone-rod dystrophy). The disease is usually progressing slowly, taking decades to fully develop the symptoms.
The type of RP which is not associated with any other organ or tissue dysfunction is called nonsyndromic RP and it is divided into three stages.

1.2.1 Nonsyndromic retinitis pigmentosa

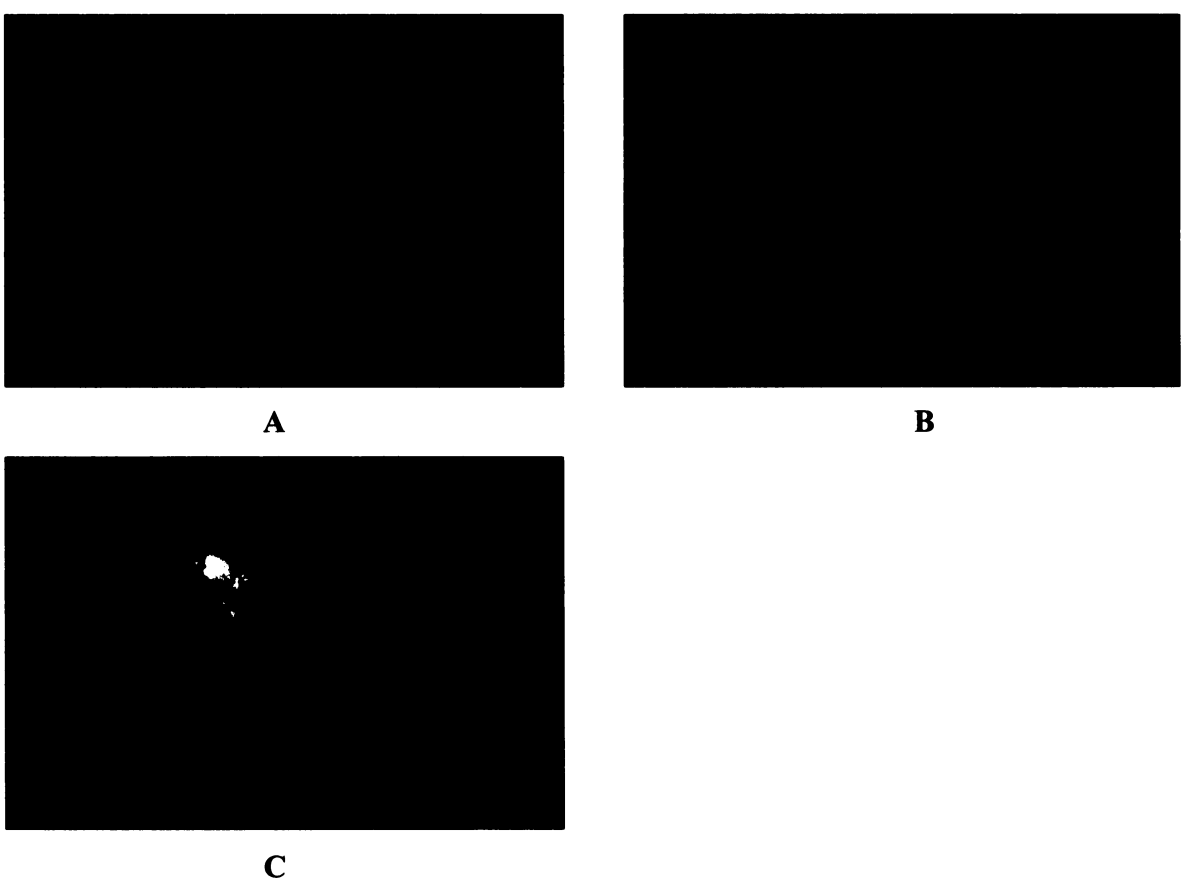
In the early stage the continuous loss of rods leads to night blindness (nyctalopia), which is often initially ignored by the patients as they do not experience any major sight difficulties in their everyday life. Slight vision field defects may occur, however these are perceivable only in dim light as well.
Even if the patient is examined by ophthalmologist the diagnosis is difficult to establish at this stage. Fundus and the optic disc appear normal. The visual field test comes out negative. Visual acuity and colour vision are both normal. Even the most powerful test - the electroretinography (ERG) may provide normally-looking output. Nonetheless this is the most reliable and objective test for diagnosis and evaluation of RP severity, based on measuring the electrical responses of the retina to flashes of light recorded by a contact electrode.
In the mid stage the symptoms are developed and the patients usually cannot ignore them any longer. Everyday activities may become very dangerous due to the loss of the peripheral visual field even in the daylight, left alone in worse light conditions. Severe loss of rod photoreceptor sensitivity is further deteriorating night blindness, which may prove life threatening (e.g. while driving at dusk or at night). In addition the patients are afflicted with photophobia - an excessive sensitivity and aversion to light, which significantly narrows the scale of brightness in which they can see normally. Many of the patients are also unable to discern pale colours.
As the photoreceptor cells degenerate and die the RPE becomes affected by the disease as well. Retinal arterioles diminish and RPE begins to degenerate in the peripheral retina. Impaired RPE cells release melanin pigment, which accumulates in typical perivascular bone spicule-shaped deposits (see figure 2). These formations and narrowed retinal vessels are observed by fundoscopic examination.

Electroretinogram is clearly abnormal, either markedly hypovolted or unrecordable at all.

Visual field testing reveals peripheral scotomas (areas or island of lost or impaired visual acuity).

In the end stage there remains only a small field of vision around the fixation point. This symptom is called the tunnel vision.

Eventually the central field of vision is lost as well. The patients are then able only to perceive light, or go completely blind. Ophthalmoscopic examination shows vast areas of pigment deposits, pallor of the optic disc and very thin retinal vessels.



A **B**

C

Figure 2: Fundus of a patient with RP, (**A**) - early stage, (**B**) - mid stage, (**C**) - end stage
Progression of expansion of typical pigment deposits and retinal vessels attenuation are obvious
Adopted from Hamel et al., 2006

1.2.2 Establishing the diagnosis

A conference in 1983 resolved upon a consensus in establishing the diagnosis [3]. The following symptoms have to be present, involving both eyes, to establish the RP diagnosis.

1. Rod dysfunction (measured by dark adaptation test or ERG)
2. Progressive loss in photoreceptor function
3. Loss of peripheral vision

The typical degenerative changes of retina (bone spicule-shaped pigment deposits, attenuated retinal vessels, loss of RPE and pallor of the optic nerve) needn't be present in order to establish the RP diagnosis, but are distinguishing it from other retinal dystrophies with similar clinical findings.

1.2.3 Syndromic retinitis pigmentosa

There are over 30 syndromes associated with RP that affect non-ocular tissues. These syndromes diagnosed in 20-30% patients with RP [1].

The most common of these syndromes is Usher syndrome, which associates with 10-20% of all RP cases [4]. This form of disease causes hearing impairment and eventually deafness.

Another frequent form of syndromic RP is Bardet-Biedl syndrome, which associates with 5-6% of RP cases [5]. The manifestations of this syndrome are obesity, cognitive impairment, polydactylism, hypogenitalism and renal structure anomalies that may lead to renal failure.

There are many other less frequent syndromes associated with RP, some of them very interesting indeed. For example the Cohen syndrome (also known as Cervenka syndrome, named after Jaroslav Cervenka [6]) caused by mutation in COH1 gene encoding a protein involved in vesicular trafficking [7]. The clinical picture of this disease (facial dysmorphism, mental retardation, short stature etc) indicates crucial role of the protein in vesicle-mediated sorting and intracellular protein transport.

1.3 Genetic profile

1.3.1 Inheritance patterns

Retinitis pigmentosa is very genetically heteregenous disease. While mutations in different genes may cause the same form of the disease, different mutations in one gene may cause different forms of RP [8] [9]. For example, different mutations in RHO gene may cause either autosomal dominant RP or autosomal recessive RP.

The inheritance pattern serves as a classification criterion. The nonsyndromic form can be inherited in an autosomal dominant, autosomal recessive, X-linked and rarely also digenic manner.

inheritance pattern	Proportion of all RP patients
autosomal dominant RP (adRP)	15-25%
autosomal recessive RP (arRP)	5-20%
X-linked RP (xlRP)	5-15%
unknown: simplex	40-50%
digenic RP	very rare

Table 1: Proportion of RP patients by mode of inheritance, Adapted from Fishman et al., 1978

Isolated cases (also simplex cases - single occurrence in a family) may be a result of *de novo* mutations, undetected autosomal recessive inheritance or disinformation on RP occurrence among relatives.

Digenic RP is very rare because there have to be two mutant forms of different genes present simultaneously in the affected person. One of them, a mutation in PRPH2 gene (L185P), is common to all the reported cases. The other one is in ROM1 gene and it is more variable [10].

Large majority of xlRP is caused by mutations in PRPG and RP2 genes. The females with xlRP are either unaffected carriers or the disease is usually much less severe in than in males.

While dominantly inherited mutations usually alter the encoded protein sequence and result in functionless or even toxic variant (termed gain-of-function mutations), the recessively inherited ones usually eliminate the encoded protein (loss-of-function mutations).

1.3.2 Causal genes

So far there are at least 48 loci identified as sites of mutations causing RP (see table 2), still accounting for only about half of all RP cases.

For most of the genes accounting for RP there are many different disease-causing mutations identified. On the other hand most of these are rather rare and there seem to be some "common" mutations in particular genes, which are frequent among the RP patients.

Most of the genes account for only small proportion of RP cases. However, there are three exceptions: the RHO gene (encoding rhodopsin), which accounts for about 25% of autosomal dominant RP (adRP), the USH2A gene (encoding membrane-associated Usher syndrome 2A

protein, probably important to development and homeostasis of inner ear and retina), which accounts for about 20% of autosomal recessive RP (arRP) and finally, the RPRG gene encoding RP GTPase regulator protein accounting for 70% of X-linked RP (xlRP). These three combined account for approximately 30% of all RP cases [1].

inheritance pattern	mapped loci (not identified)	mapped and identified genes
autosomal dominant RP	RP33	CA4, CRX, FSCN2, GUCA1B, IMPDH1, NR2E3, NRL, PRPF3, PRPF8, PRPF31, PRPH2, RHO, ROM1, RP1, RP9, SEMA4A, TOPORS
autosomal recessive RP	RP22, RP25, RP28, RP29, RP32	ABCA4, CERKL, CNGA1, CNGB1, CRB1, LRAT, MERTK, NR2E3, NRL, PDE6A, PDE6B, PRCD, PROM1, RGR, RHO, RLBP1, RP1, RPE65, SAG, TULP1, USH2A
X-linked RP	RP6, RP23, RP24, RP34	RP2, RPGR

Table 2: Genes accounting for particular forms of RP, adapted from RetNet, for further details on particular genes see Entrez Gene at NCBI or GeneCards

1.4 Affected biochemical pathways

The most common RP mutations occur in genes encoding proteins involved in phototransduction (the process by which the energy of a photon is converted into a neuronal signal). These are: rhodopsin (RHO), α and β subunits of cyclic guanosine monophosphate phosphodiesterase (PDE6A, PDE6B), α subunit of cyclic nucleotide gated channel (CNGA1) and arrestin (SAG).

A group of proteins is involved in recycling of 11-cis-retinaldehyde (chromophore absorbing light at the beginning of phototransduction cascade). These are: ATP-binding cassette transporter of rods (ABCR), cellular retinaldehyde binding protein (CRalBR), retinal pigment epithelium-specific protein 65kDa (RPE65) and RPE G-protein coupled receptor (RGR). Proteins of this group are predominantly expressed in RPE.

Mutations in genes encoding regulators of gene expression at transcriptional and posttranscriptional levels are also associated with RP. All RP associated transcription factors

known so far are photoreceptor-specific. These are for example neural retina leucine zipper (NLR) or cone-rod homebox (CRX).

In contrast with these trascription factors, RP associated RNA processing factors (four adRP associated pre-mRNA splicing factors), are all expressed ubiquitously. These splicing factors are the focal point of this paper and will be described in detail thereinafter.

Furthermore, there are some photoreceptor cell structural proteins disordered due to mutations in their respective genes. These are: peripherin 2 (PRPH2), retinal outer segment membrane protein 1 (ROM1), fascin homolog 2, retinal actin bundling protein (FSCN2) and prominin 1 (PROM1).

1.5 Treatment

So far there is no medication or surgery to treat RP. Although there were some studies indicating that oral supplements of vitamin A slow the course of the disease [11], the results are rather disputable and it would require a larger clinical trial to assess the effectiveness of the treatment.

Thus the patients can only hope for development of novel treatments. There are some promising approaches showing good results in animal experiments and even in human trials. There seems to be huge potential in stem cells therapy using limbal epithelium derived neural progenitors [12].

Another evolving field is gene therapy, which would make the best of the knowledge in the causal genes. However the clinical potential of this approach is limited due to the vast range of mutations causing RP.

There are new drugs already being tested on human RP patients (e.g. ciliary neurotrophic factor released from special cell capsule implants [13]) or nilvadipine [14]. These neuroprotective drugs have a considerable advantage compared to the gene therapy - they are applicable on wide range of disease-causing mutations.

There is a remarkable progress in the field of electrode retinal prosthesis development, which would return sight to the patients by technical means [15].

2. Pre-mRNA splicing

2.1 Pre-mRNA processing

The precursor messenger RNA is transcribed from DNA by RNA polymerase II and undergoes co-transcriptional and post-transcriptional processing, before it is exported from the nucleus. There are several possible RNA modifications in eucaryotes. Three of them are crucial for precursor messenger RNA (pre-mRNA) maturation.

The first modification of pre-mRNA is 5' end capping. This process begins as soon as only about 25 nucleotides long nascent pre-mRNA chain has been synthesized. The capping reaction itself is performed by three enzymes in subsequent steps. At first a phosphate from 5' end of the nascent RNA is removed by a phosphatase. Then a GMP (from GTP) is added by guanyl transferase via 5' to 5' linkage. Finally, a methyl group is added to the guanosine by methyl transferase.

Other essential processing step in pre-mRNA maturation is 3' end polyadenylation. In this process, the nascent RNA chain is cleaved by an endonuclease complex associated with RNA polymerase II C-terminal domain (CTD), after transcription of the polyadenylation signal. Immediately after the cleavage, polyadenylate polymerase starts to add adenosine residues to the free 3' end, creating a poly-A tail consisting of up to 250 adenosines.

These two modifications are vital to export of mature mRNA from the nucleus, stability of the mRNA molecule and its translation in the cytoplasm. The 5' cap also promotes the excision of 5' end proximal noncoding intervening sequence (intron) [16].

A typical mammalian pre-mRNA is abundant in introns that have to be excised to form mature functional mRNA. These noncoding sequences actually represent 90% of unspliced pre-mRNA molecule on the average. Removal of introns and joining of interrupted coding sequences (exons) is performed through the process of pre-mRNA splicing.

2.2 The splicing cycle

In this part I will concentrate on the major type of splicing in eucaryotes and neglect the minor ones like self splicing introns (in transcripts of some mitochondrial and chloroplast genes) and AT-AC splicing (approximately 0.1% of introns are spliced this way in humans).

The excision of introns and joining of exons takes place in an immense ribonucleoprotein complex termed spliceosome. There are five essential subcomplexes of the spliceosome called

small nuclear ribonucleoproteins (snRNPs), each of them containing a small nuclear RNA (snRNA) U1, U2, U4, U5 or U6. The snRNAs are almost certainly the main catalytic agent of the spliceosome, forming its catalytic centre only after assembly and rearrangement of the splicing components on mRNA [17].

In addition to these complexes, splicing also requires many non-snRNP protein factors. Advanced methods of spliceosome purification and mass spectrometry analysis revealed about 300 different proteins included in the spliceosome, which classifies the spliceosome as one of the most intricate macromolecular complexes known [18].

There are three hypotheses explaining why such an extraordinary amount of proteins is needed, while the catalytic activity is performed mainly by snRNA. The first one suggests that many of these factors are needed for accuracy of the splicing process, inspecting splicing signals and performing proofreading activity. There really are some energy-dependent checking mechanisms verifying the assembly of spliceosome before and after the first transesterification reaction [19]. Processes like this one may be more abundant, thus contributing to the complexity.

The second hypothesis points out that many of the proteins probably are not necessary for splicing itself, but rather play a vital role in post splicing processes. For example proteins of the exon junction complex play important roles in mRNA nuclear export, subcellular localization and translational yield enhancement [20].

The third theory suggests that some of the proteins are involved in physical linking of snRNPs with RNA polymerase II during cotrascriptional splicing [21].

It is probable that all of these theses are more or less true and contribute to the whole picture. Nonetheless, defining exact roles of all proteins involved in these processes remains a challenge.

2.2.1 Splice site recognition

Introns have to be excised precisely in order to avoid shifts in open reading frame (ORF) after exon joining, which would result in a useless mRNA encoding a nonsense protein. Therefore, there has to be a foolproof mechanism ensuring high fidelity in splice-site recognition. This is managed by several specific networks of RNA-protein, protein-protein and RNA-RNA interactions. These interactions take place at four pre-mRNA sequence elements: the 5' exon-intron splice site, the branchpoint sequence (BPS), the pyrimidine-rich tract (py tract) and the 3' intron-exon splice site (see figure 3).

In addition, there are sequences contained in exons that either enhance (exonic enhancers) or inhibit (exonic inhibitors) splicing through interaction with protein splicing factors. This plays a vital role in alternative splicing.

The 5' exon-intron splice site is marked by the consensus sequence AG↓**GU**RAG (R = purine, Y = pyrimidine, ↓ marks the actual splice site, highly conserved nucleotides are bold). The end of the intron, the 3' splice site, is marked by Y**AG**↓RNNN. About 30 nucleotides upstream of the 3' splice site lies the BPS - CURA^{2OH}Y, which is followed by py tract [22].

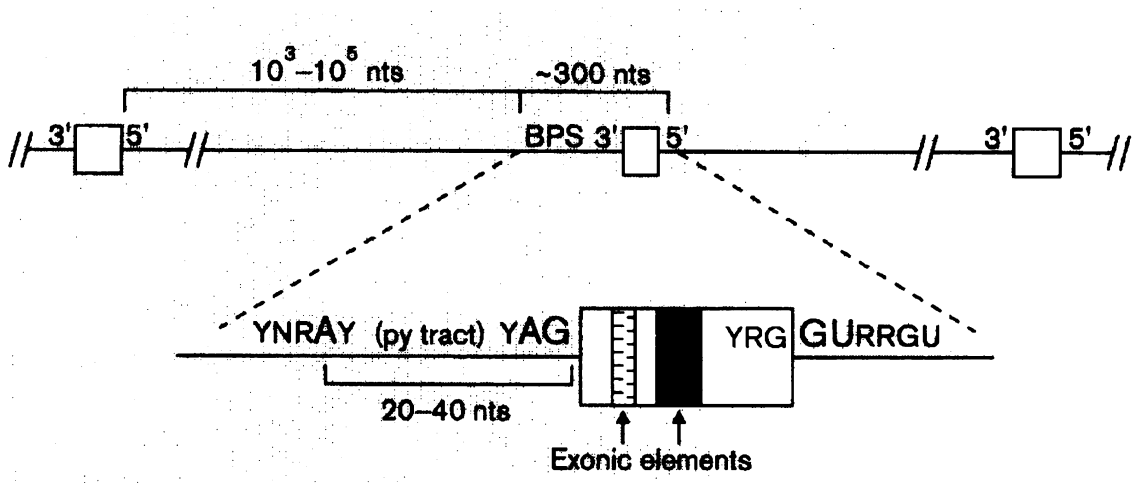


Figure 3: Scheme of splice-site recognition determinant sequence elements
Adopted from Reed 1996

There is a model of the intricate network of interactions in splice sites recognition (respectively, forming of the early spliceosome complex) based on studies of artificial pre-mRNA containing only one short intron and optimal splice sites sequences. These studies allowed us to gain deep insight into the process and respective roles of its participants (see figure 4).

However, typical human mRNA contains multiple large introns (10^3 - 10^4 nucleotides), small exons (less than 200 nucleotides on average) and weakly conserved splice sites [23]. Initial splice sites in these mRNAs are recognized across the exons as exonic pairs, when the intron length exceeds 250 nucleotides [24].

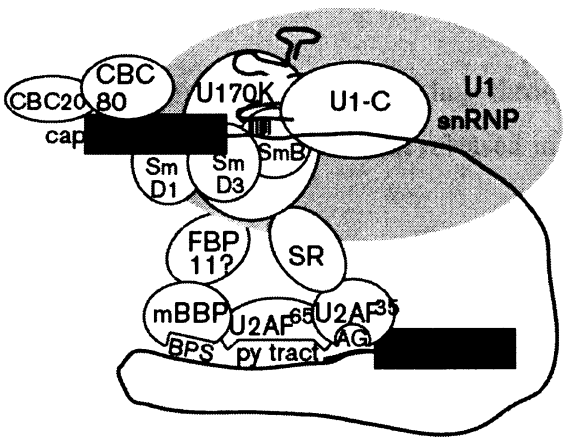


Figure 4: Scheme of interactions in splice-sites recognition, Adopted from Reed, 2000

In this model Serine/Arginine-rich proteins (SR proteins) are binding to exonic enhancers and U1/U2 components are binding on the fringes of the exon on the nascent pre-mRNA (see figure 5). Individual heterogenous nuclear ribonucleoproteins (hnRNPs) may bind to the exon as well, while stable hnRNP complexes that compete with spliceosomal assembly by encompassing long pre-mRNA sequences (about 500 nucleotides), are left to bind on the long intron sequences [25].

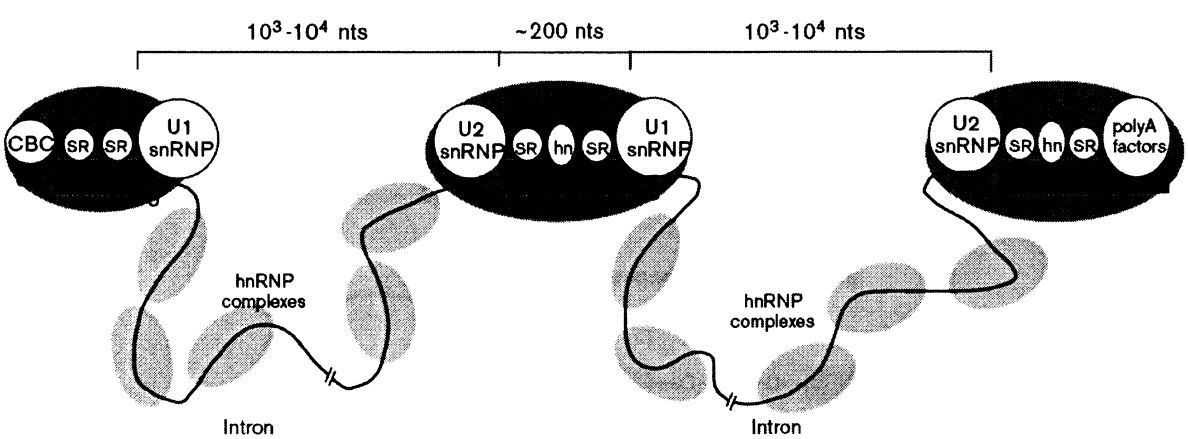


Figure 5: Scheme of initial splice-site recognition across exons, Adopted from Reed 2000

The spliceosomal components assembled across the exon (U1 snRNP and U2 snRNP) are linked to adjacent exons in order to juxtapose them and continue the spliceosomal assembly [26].

2.2.2 The splicing reaction per se

The splicing reaction itself is a two-step process proceeding through two transesterification reactions. Products of these reactions are: excised intron released in the form of lariat (a loop structure - see figure 6) and two joined exons.

In the first step, the 2' hydroxyl of the conserved adenosine located in the BPS carries out a nucleophilic attack on the 5' splice site, forming a 2'-5' phosphodiester linkage with the 5' terminal nucleotide of the intron, thus creating the lariat. The products are: free 5' exon (still encompassed in the spliceosomal complex) and intron-3' exon.

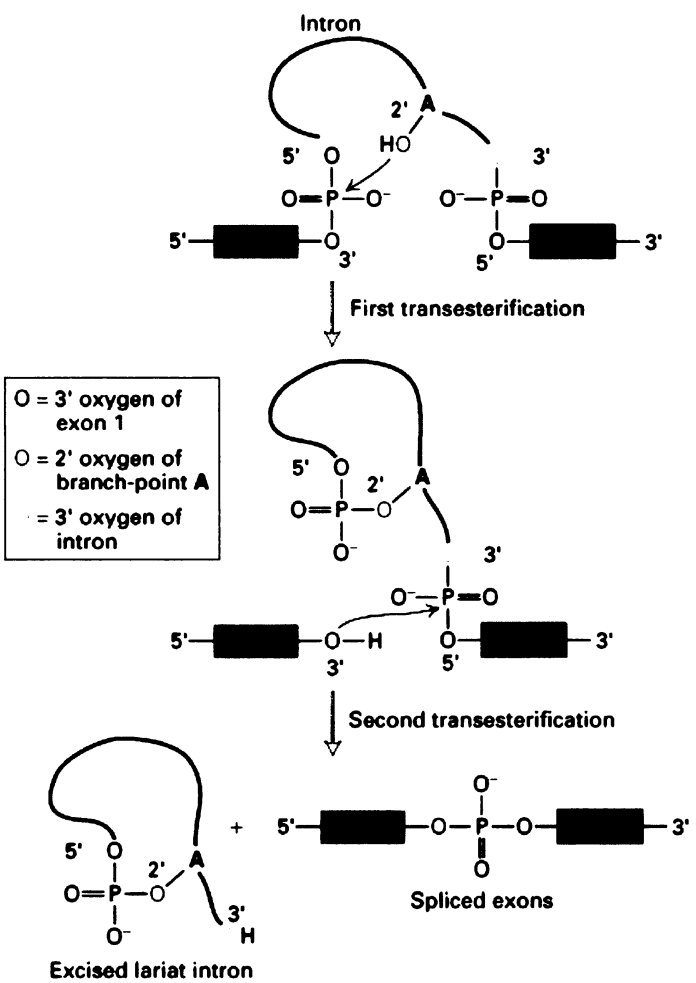


Figure 6: Chemistry of the splicing reaction, Adopted from http://departments.oxy.edu/biology/Stillman/bi221/111300/processing_of_hnrnas.htm

After conformational changes in the spliceosome, the second step takes place. This time, the nucleophilic attack is carried out by 3' hydroxyl of the 5' exon, which was created in the first

step, aiming for the 3' splice site and forming an exon-exon bond. The intron is then released and spliceosome disassembles.

2.2.3 Spliceosome assembly

Interaction of the U1 snRNP with the 5′ splice site initiates spliceosome assembly. This interaction is performed by base pairing of U1 snRNA with 5' splice site sequence and has to be stabilised by associated proteins, as there are only four to seven base pairs established. These proteins are schematically depicted in figure 4. Some of these proteins also participate in 5' splice site recognition via their RNA-binding motifs (some members of the SR protein family).

Branch point sequence is recognized by branch point binding protein (BBP), which interacts with heterodimerous U2 auxiliary factor (U2AF), whose subunits bind to py tract and 3' splice site AG [27] [28].

These two complexes (at 5' splice site and 3' splice site) are interconnected by other protein factors (namely members of the SR family [29]), thereby forming the early complex (E complex).

Completion of E complex assembly promotes binding of U2 snRNA to the BPS along with its associated U2 snRNP proteins, that bind upstream and downstream of the BPS stabilizing the interaction analogously to U1 snRNP proteins. Six U2 snRNP proteins binding to pre-mRNA are subunits of essential splicing factors SF3a and SF3b [30].

Binding of U2 snRNP to the BPS is ATP dependent and represents a key step in the splicing cycle as it bulges out the conserved adenosine from the BPS to an extrahelical position important for the first transesterification reaction [31].

After addition of U2 snRNP the pre-spliceosomal complex is termed A complex. Subsequently, the U4/U6•U5 tri-snRNP binds at the 5' splice site, thus forming the B complex. Extensive RNA-RNA and RNA-protein rearrangements then occur. The duplex of U4 and U6 snRNAs is unwound, which results in the release of U4 snRNP. Base pairing at the 5' splice site is dissociated as well and U1 snRNA is replaced by U6 snRNA, which results in the release of U1 snRNP (see figure 7). U6 snRNA also forms long base pairing with U2, which brings the 5' splice site and the BPS in close proximity.

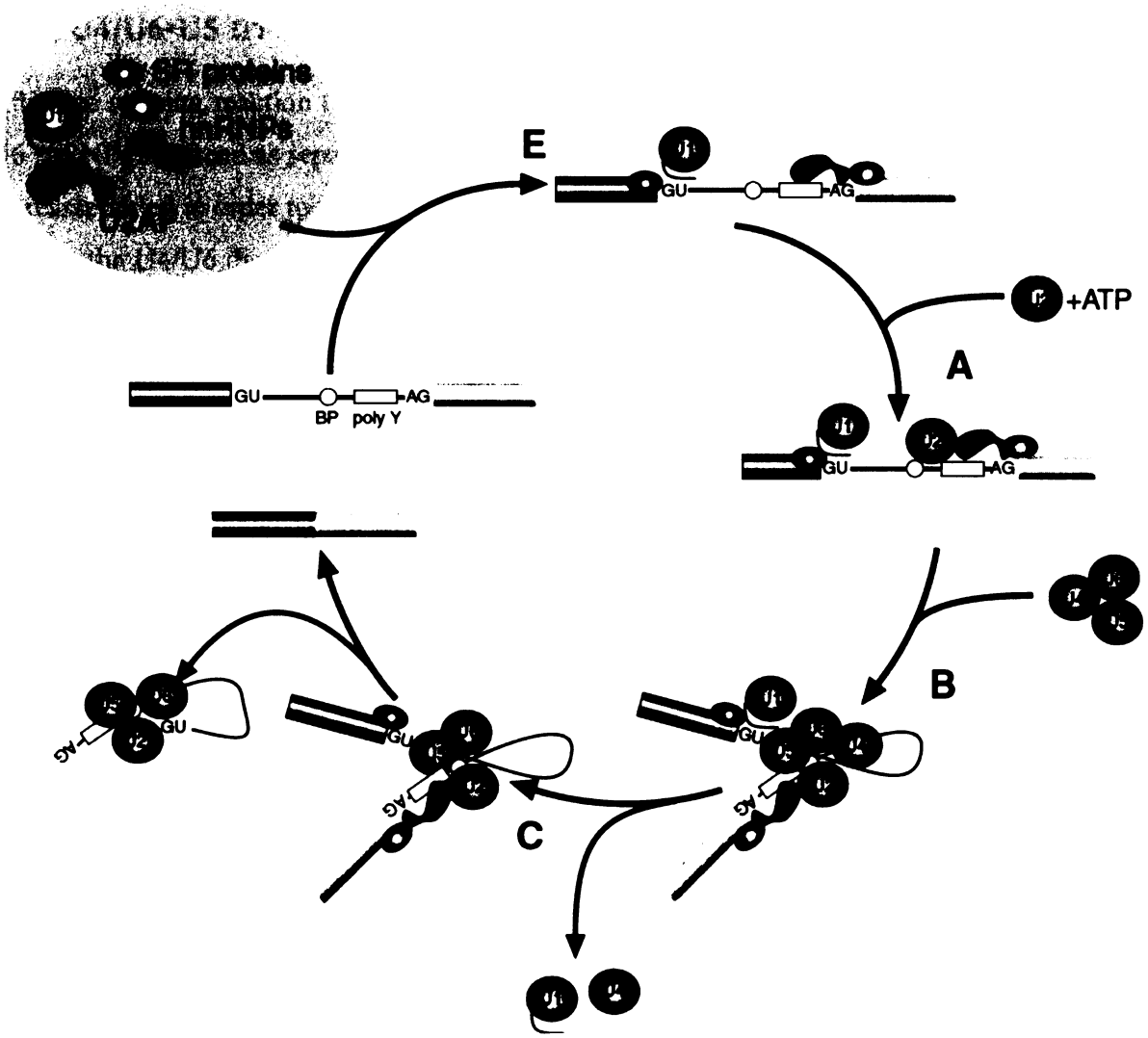


Figure 7: Scheme of spliceosome assembly, Adapted from Sanford et al., 2004

These ATP driven rearrangements are crucial for formation of the catalytic centre and performing the first transesterification reaction and are termed spliceosomal activation. Rearranged U6-U5-U2 spliceosome with active catalytic centre is termed C complex.

The role of U5 snRNA is probably rather in positioning of the exons for the second transesterification reaction, while U2 and U6 snRNAs are supposed to be the main constituents of the catalytic centre [17].

It remains a question, whether the spliceosome is really assembled in this stepwise manner or is prefabricated as indicated by Nilsen et al. [32].

2.2.4 U4/U6•U5 tri-snRNP recycling

After the splicing reaction the snRNA base pairing is unwound by helicases and U2, U5 and U6 snRNPs are freed as separated complexes [33]. Therefore, the U4/U6•U5 tri-snRNP has to be reassembled in order to undergo next splicing cycle.

At first the U4/U6 di-snRNP is reassembled. This is triggered by U6 snRNA 3' end terminal uridine stretch phosphorylation, which results in formation of a heteroheptameric ring complex consisting of LSm2-8 proteins replacing the La protein at the 3' end and subsequent transient binding of p110 (SART3) protein, which promotes di-snRNP recycling that probably takes place in Cajal bodies [34] [35] [36].

Cajal bodies are subnucear structures higly enriched in snRNPs, where maturation of newly synthesized snRNPs takes place [37]. Other subnuclear structures that are enriched in pre-mRNA processing factors, including splicing factors are the speckles [38].

How the pre-mRNA processing factors are targeted to these subnuclear structures and what triggers their release remains disputable. It is also possible that there is no such targeting and that these proteins move just by Brownian motion and interact with binding sites concentrated in these subnuclear structures coincidentally [39].

The association of U5 snRNP with the di-snRNP is managed via protein-protein interactions that will be described thereinafter.

2.3 Alternative splicing

Transcripts of vast majority of human genes (approximately 70-80% [40]) are spliced in multiple different ways. This variability in splicing greatly expands the information content and regulation possibilities of the human genome, allowing structurally and functionally distinct protein isoforms to be encoded by a single gene.

This is managed by several forms of pre-mRNA alternative splicing including optional intron inclusion, optional exon exclusion, use of alternative splice sites etc. Regulation of alternative splicing is highly tissue and developmentally specific [41]. The use of alternative splice sites is regulated, among other mechanisms, by protein factors (RS and hnRNP proteins) that bind to either exonic or intronic splice site enhancer and silencer sequence elements [42]. In addition, it is also influenced by: splice site strength (complementarity with U1 snRNA and py tract length), size of introns and exons, pre-mRNA secondary structure and processivity of RNA polymerase II [24].

Many human diseases are caused by malfunctioning alternative splicing, which results in expression of aberrant protein isoforms.

3. Pre-mRNA splicing and retinitis pigmentosa

There are many distinct mutations in several genes accounting for RP that affect pre-mRNA splicing. These mutations are of two kinds: cis-acting mutations that alter splicing of the respective pre-mRNAs by modifying constitutive or alternative splice sites within them, creating cryptic splice sites or affecting pre-mRNA secondary structure, and trans-acting mutations that affect components of the splicing machinery, thus causing impaired pre-mRNA splicing [43]. Aberrant splicing is supposed to be the molecular mechanism underlying up to 60% of disease-causing mutations in humans [44].

There are several splice-site mutations identified in RP patients. For example: splice-site mutations in RHO gene [45], xlRP caused by 5' splice site mutation in RPGR gene [46] or arRP caused by defective splicing of PDE6B gene transcript [47].

As mentioned in the first chapter, there are four adRP associated genes encoding pre-mRNA splicing factors. Trans-acting mutations in these genes account for approximately 9.5% of adRP cases [48].

These splicing factors are: pre-mRNA processing factor 31 homolog - hPrp31 (also known as RP11, PRP31, PRPF31, 61 kDa U4/U6), pre-mRNA processing factor 3 homolog - hPrp3 (also known as RP18, PRP3, PRPF3, 90 kDa U4/U6), pre-mRNA processing factor 8 homolog - hPrp8 (also known as RP13, PRP8, 220 kDa snRNP, PRPF8) and Retinitis pigmentosa 9 protein - RP9 (also known as PAP-1, PIM-1 associated protein). All these proteins are highly conserved in the evolution, which indicates their essential function during splicing

The hPrp8, hPrp31 and hPrp3 are essential in formation of U4/U6•U5 tri-snRNP and its recruiting to catalytically active spliceosome (see figure 8), thus in pre-mRNA splicing in general. RP9 is also involved splicing - it interacts with hPrp3. The roles of respective factors will be discussed in detail thereinafter.

It was also mentioned in the first chapter that unlike any other RP associated gene, these are all expressed ubiquitously. This implicates perhaps the most intriguing question concerning these splicing factors: How is it possible that malfunction of these essential components of the spliceosome, thus proteins necessary for cell survival, leads to a cell-type specific disease that is not life-threatening?

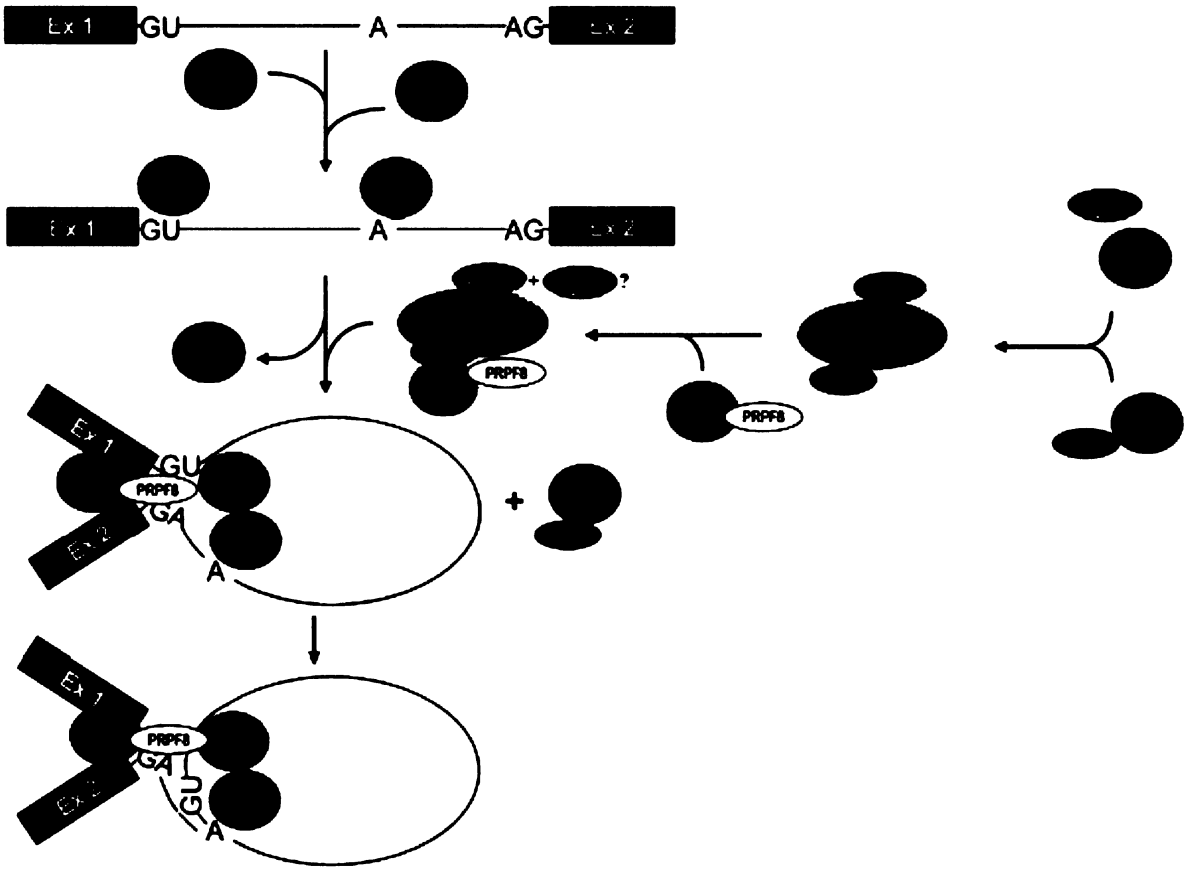


Figure 8: Scheme of function of adRP associated splicing factors in spliceosome assembly
Adapted from Mordes et al., 2006

There are several models proposed to explain why only photoreceptor (and secondarily also RPE) cells are affected by these mutations.

The first two models assume that photoreceptor cells have extraordinary high demand on mRNA and protein production, thus also on splicing, compared to other cell types due to the continuous membrane disks creation and shedding in their outer segments. Therefore, the loss of one functional allele of these splicing factors leads to insufficiency of splicing and slow degeneration of photoreceptor cells. This is called the haploinsufficiency model (see figure 9). The second model takes into account that all the adRP associated splicing factors participate in tri-snRNP formation and function. Impaired function of these factors may result in decreased rate of spliceosomal assembly, thus making splicing the limiting step in protein synthesis. This could bring the cell into a vicious circle as higher expression of spliceosomal components cannot substitute the function of these factors due to the decreased rate of splicing.

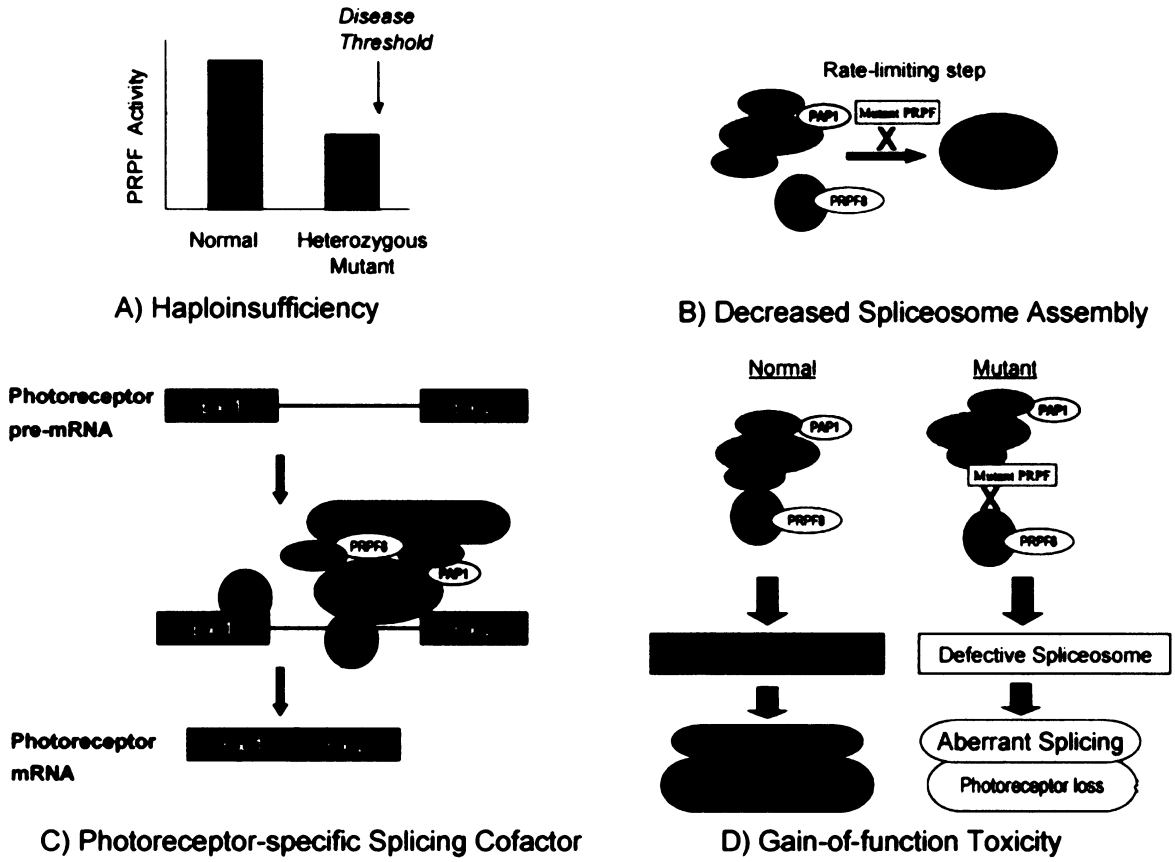


Figure 9: Proposed models of mechanisms causing photoreceptor-specific phenotype of adRP associated splicing factors mutations, Adopted from Mordes et al., 2006

There could also be a substantial role of some putative photoreceptor-specific cofactors that interact with these splicing factors and are the true causes underlying the photoreceptor-specific manifestations of the disease.

The fourth model assumes that some mutations altering the function of these splicing factors may cause a gain-of-function toxicity in the photoreceptor cells as a result of aberrant splicing and expression of some photoreceptor-specific genes or as a result of accumulation of functionless mutant form of the splicing factor itself.

Another possibility (not depicted in figure 9), is a putative photoreceptor-specific function unrelated to splicing that could be performed by these factors.

There is a study suggesting that there may be a substantial role of physiological changes resulting from cyclical fluctuation of oxygen level at the outer retinal layers due to the circadian light/dark cycle [49]. These changes alter the expression of three ischemia-hypoxia response genes encoding U4/U6•U5 tri-snRNP associated splicing factors: PRPF4 encoding di-snRNP component hPrp4, SART1 encoding tri-snRNP component hSnu66 (also 110 kDa,

HAF) that also acts as a transcription factor for EPO gene encoding erythropoietin [50], which has degeneration-protective effect on photoreceptors [51], and finally, LSM8 encoding LSm8 U6 snRNA associated protein that participates in di-snRNP recycling (see chapter 2). Another oxygenation-regulated gene is PIM-1 encoding Pim-1 kinase. Its putative role in pre-mRNA splicing will be described thereinafter.

This is just a summarization of the proposed models and it is probable that different mutations cause the disease via distinct mechanisms, or even via mechanisms nobody defined yet, as there are very few of these mutations biologically characterized so far.

Let's have a closer look on the respective adRP associated splicing factors and see whether there is some evidence supporting some of the proposed models.

3.1 adRP associated splicing factors

3.1.1 RP9

The RP9 gene (MIM *607331) maps to chromosome 7q14.3, contains six exons and was identified as accounting for adRP type 9 by Keen et al. [52]. It encodes 221 amino acid residues (aa) long PAP-1 protein (UniProt Q8TA86) with molecular weight of 26.1 kDa.

Its function remains unknown, but there are several interactions with other proteins described, that may serve as a clue.

PAP-1 was identified as a Pim-1 kinase binding protein, although it is not phosphorylated by Pim-1 *in vivo* [53]. Pim-1 is an oncogene participating in suppression of apoptosis and cell cycle progression [54] and probably plays a role in control of PAP-1 phosphorylation which seems to determine its localization in splicing speckles in the nucleus [53].

PAP-1 was also found to directly interact with U2AF1 (U2AF 35 kDa), which is a 3' splice site recognition protein, and with hPrp3 C terminal region, indicating that its role may be in bridging the interaction between these two splicing factors. It was also revealed that PAP-1 is weakly associated with U4/U6•U5 tri-snRNP [55].

There are two mutations in RP9, both described by Keen et al. [52]. The first one is missense mutation His137Leu caused by transversion in codon CAT-CTT in exon 5. The second one is also missense change Asp170Gly caused by transition in codon GAT-GGT in exon 6. Neither of these mutations impairs binding of RP9 to hPrp3 [55].

Mechanism of pathogenicity of these mutations is unknown. Actually, some authors dispute the pathogenicity of mutant PAP-1 as a cause of RP9 at all [48].

3.1.2 PRPF 3

The PRPF3 gene (MIM *607301) maps to chromosome 1q21.1, contains 16 exons (Ensembl) and was identified as accounting for adRP type 18 by Xu et al. [56]. It encodes 683 aa long protein (UniProt O43395) weighing 77.5 kDa.

Unlike RP9, the function of hPrp3 is quite well characterized. It is a U4/U6 specific splicing factor vital to U4/U6 di-snRNP stability, formation of U4/U6•U5 tri-snRNP and possibly also to addition of U4/U6•U5 tri-snRNP to the spliceosomal A complex. In order to perform these functions it interacts with several proteins and also with snRNA (see figure 10).

It forms a heterotrimeric complex with hPrp4 and 20 kDa cyclophilin H (PPIH) associated with U4/U6 di-snRNP [57] [58]. It also interacts with tri-snRNP specific proteins hSnu66 and hPrp6 [59].

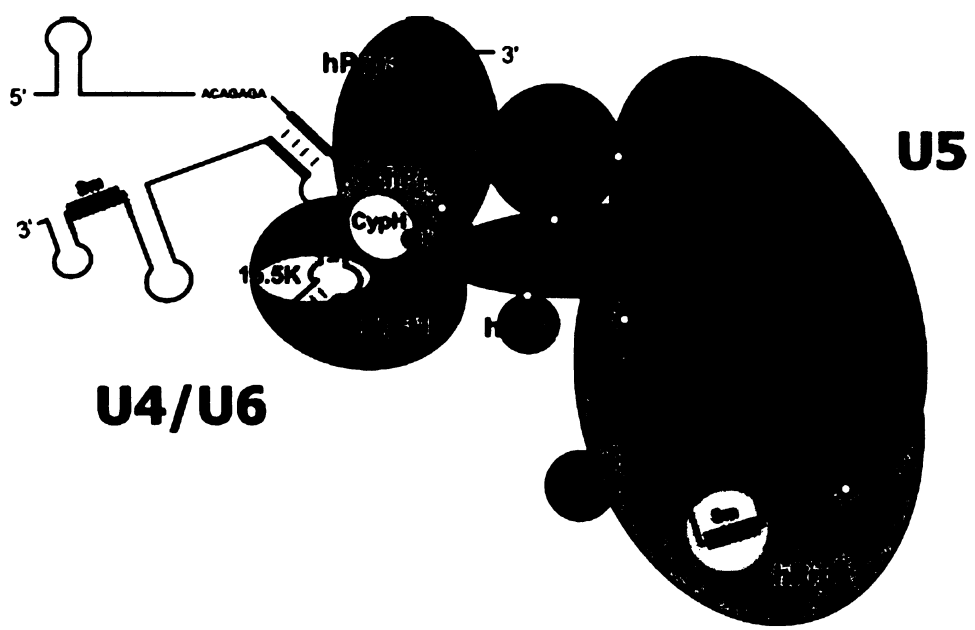


Figure 10: Model of some protein-protein interactions within the tri-snRNP (marked with dots), Adopted from Liu et al., 2006

It also interacts with SPF30, which bridges the interaction between U2AF1 and U4/U6•U5 tri-snRNP, which may be an essential step in addition of tri-snRNP to the A complex [60]. As mentioned above, PAP-1 also bridges an interaction between U2AF1 and hPrp3, thus contributing to putative importance of this linkage.

After rearrangements in the spliceosome and unwinding of U4 and U1, hPrp3 also leaves the complex (along with other U4/U6 proteins, U2AF1, U2AF2 and SPF30) [61].

A recent paper revealed that hPrp3 also binds to casein kinase 2 (CK2), with which it colocalizes in nuclear speckles, and that its C terminal domain is also phosphorylated by CK2 both *in vivo* and *in vitro* [62]. This paper also shows that phosphorylation of hPrp3 by CK2 promotes overall splicing. CK2 is involved in cell cycle control, signalling, intracellular trafficking, transcriptional and posttranscriptional processes [63] [64].

The hPrp3 protein is highly conserved in the evolution, especially its C terminal region [65], which is crucial for this phosphorylation and for interactions with PAP-1 and hPrp6. All three known adRP associated missense mutations of PRPF3 are very close to each other in exon 11 in this highly conserved C terminal region.

The first one is Thr494Met caused by transition in codon ACG-ATG described by Chakarova et al. [66]. The second one is Pro493Ser caused by transition in codon CCC-TCC also described by Chakarova et al. [66]. The third one is Ala489Asp caused by transversion C-A described by Gamundi et al. [67].

Unlike sporadic Pro493Ser, the Thr494Met mutation is common to several unlinked RP families worldwide and more importantly; there has been significant progress in understanding the molecular basis of its pathogenicity. Due to the absence of Thr494 the C terminal region of hPrp3 cannot be properly phosphorylated, which impairs the function of the whole factor. The mutation does not affect PRPF3 expression; however it weakens the association of hPrp3 with hPrp6, hPrp4, U4 and U6 snRNAs and leads to reduced cell proliferation and viability [68].

In addition, this mutation also affects nuclear distribution of hPrp3 and causes forming of big aggregates, which leads to apoptosis specifically in photoreceptor cells [69]. In contradiction with the model proposed by Ghonzales-Santos et al. this paper also suggests that there's rather a cell-specific toxic gain-of-function effect of hPrp3 Thr494Met mutation than haploinsufficiency that causes the disease, which is supported by a recent paper by Graziotto et al. [70].

3.1.3 PRPF8

The PRPF8 gene (MIM *607300) maps to chromosome 17q13.3, contains 42 exons and was identified as accounting for adRP type 13 by Greenberg et al. [71]. It encodes a huge 2335 aa long protein (UniProt Q6P2Q9) with molecular weight of 273.6 kDa.

The hPrp8 protein plays a crucial role in splicing as it is necessary for U4/U6•U5 tri-snRNP formation. Moreover, it forms the catalytic centre of the active spliceosome probably

positioning the U5 and U6 snRNAs in it. It also interacts with a broad range of essential splicing factors and pre-mRNA at all splice-site recognition sequence elements [72] [73].
There are 13 mutations (Human Gene Mutation Database - HGMD) described in PRPF8 that cause severe forms of adRP. Most of them are missense mutations described by McKie et al. [74]. Pretty much like in PRPF3 all these mutations (along with some deletions described by Martinez-Gimeno et al. [75]) are clustered in the last exon encoding C terminal region of hPrp8, which is especially conserved in the evolution.
The 3D structure of hPrp8 C terminal region has been resolved by Pena et al. [76]. These authors also found that mutations in this domain impair protein-protein interactions with Brr2 (helicase involved in spliceosomal activation and disassembly [77]) and Snu114 (GTPase regulating spliceosomal activation and disassembly [77]). In yeast this results in accumulation of immature U5 snRNP lacking Brr2 in the nucleus and slow splicing rate [78].
Molecular basis of pathogenicity of hPrp8 mutant forms in humans remains unknown.

3.1.4 PRPF31

The PRPF31 gene (MIM *606419) maps to chromosome 19q13.42, contains 14 exons and was identified as accounting for adRP type 11 by Al-Maghtheh [79]. It encodes a 499 aa long protein (UniProt Q8WWY3, 3D structure model at RCSB PDB - ID 2ozb) with molecular weight of 55.5 kDa.
The hPrp31 protein is a U4/U6 specific protein essential to U4/U6•U5 tri-snRNP assembly. It is required for interaction between U4/U6 di- snRNP and U5 snRNP as it binds to hPrp6 (see figure 10) [80]. With its Nop domain (InterPro IPR002687, PDB ID 1e7k), hPrp31 also interacts with hSnu13 (15.5 kDa) bound to specific U4 snRNA sequence element [81].
Interestingly, hPrp31 and hPrp3 contact the same region of U4 and U6 snRNAs contained in U4/U6 stem I [82]. Both these factors leave the spliceosomal complex after its rearrangement and activation.
There are at least 45 mutations described in PRPF31 (HGMD). There has been big effort to characterize these mutations as mutant forms of PRPF31 are the second most frequent cause of adRP [48].
Some studies like the ones by Mordes et al. [83] and Yuan et al. [84] indicated that mutant forms of hPrp31 cause defective splicing of photoreceptor specific genes (e. g. RHO, FCN2, RDS), thus explaining the cell-specific cytotoxic effect. However, a recent paper by Rio Frio et al. [85] shows that these studies are rather irrelevant. Transfection with expression vectors

containing mutant PRPF31 cDNA in order to simulate its effect *in vivo* causes aberrant splicing of photoreceptor specific genes indeed. However, this effect is just an artefact as the studied mutations actually lead to premature termination codons establishment in pre-mRNA, which is consequently degraded by nonsense mediated decay.

Nonsense mediated decay is a quality check mechanism that provides safeguard against potentially toxic products of mRNA containing premature nonsense codons [86].

Actually, most of the PRPF31 mutations (at least 35) described so far lead to premature nonsense codons establishment and cause negligible mutant allele expression and significantly decreased levels of wild-type hPrp31 protein [85]. This decrease in hPrp31 amount may have similar effect as its RNAi knockdown, which leads to impaired U4/U6•U5 tri-snRNP formation and accumulation of U4/U6 di-snRNPs in Cajal bodies, thus confirming its critical role in tri-snRNP formation [87].

These data are consistent with older results by Rivolta et al. [88] proving that higher levels of wild-type allele expression correlate with lower penetrance (severity) of the disease, and indicate that majority of PRPF31 mutations cause RP via haploinsufficiency, nonetheless not elucidating the photoreceptor specificity of the phenotype.

Two missense mutations in exon 7 have been functionally analysed as well. These are: the AD29 mutation Ala216Pro caused by G646C transversion and the SP42 mutation Ala194Glu caused by C581A transversion. These mutations cause impaired translocation of hPrp31 into the nucleus, thus causing its insufficiency, which hinders splicing [89].

The SP42 mutation significantly weakens binding to hPrp6 and identifies the coiled-coil domain of hPrp31 (see figure 11) as the interaction site for hPrp6 binding [81]. Hence, this mutation can impair the formation of tri-snRNP and its stability.

Whereas the SP42 mutation impairs interaction with PRPF6, the AD29 strengthens it, which may stabilise the tri-snRNP but on the other hand, it may lead to impaired spliceosomal activation and tri-snRNP recycling [90]. It is also presented in the same study that this mutation most probably does not negatively affect splicing of photoreceptor-specific genes, which was considered as another mechanism involved in the phenotypic manifestation.

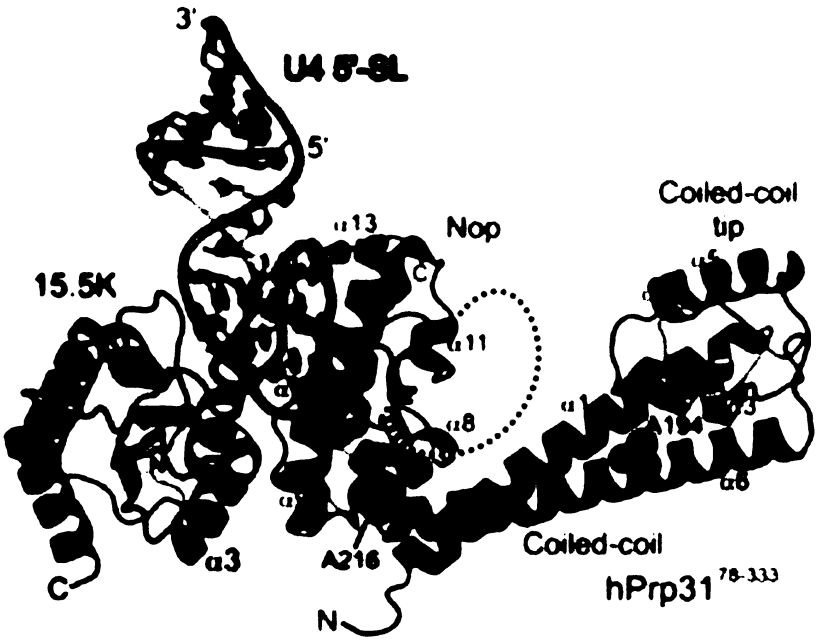


Figure 11: 3D model of hPrp31 structure (residues 78-333) and interaction with hSnu13 and U4 snRNA; sites of AD29 and SP42 mutations are depicted in cyan
Adopted from Sunbin Liu et al., 2007

Discussion

It is obvious from the presented data that there is hardly a universal disease-causing mechanism for all mutations in adRP associated splicing factors.

There is substantial evidence that most of the mutations of PRPF31 result in significantly decreased level of hPrp31 in the nucleus, which negatively affects splicing efficiency as well as do the two missense mutations studied so far. On the other hand, it seems that mutations of PRPF3 rather have a gain-of-function toxicity effect. Mutations of PRPF8 and RP9 are virtually uncharacterised.

Regarding the cell-specific cytotoxic effect, most authors accept the high metabolic demand of photoreceptors as its cause. In my opinion, this cannot be the only mechanism underlying the effect, as there sure are other highly metabolically active tissues in the human body that would be affected as well. I think, there would have been at least a later onset of some symptoms described in these tissues in patients with severe rapidly progressing RP.

Other possible mechanisms, which may contribute to this cell specificity is oxygenation level fluctuation during the circadian light cycle, and impaired trafficking and recycling of tri-snRNP components, which may have specific effect on photoreceptors as a result of subnuclear structure reorganization during neuronal differentiation [91]. Both these newer models are very interesting and deserve further investigation.

There is also hypothetical role of some unknown photoreceptor-specific splicing factor or role of yet unidentified adRP gene, as there are still about 40% cases of adRP not linked to any known mutation [48].

It seems rather improbable that there is a significant effect of mutations of adRP associated splicing factors on alternative splicing of retina-specific genes, however there are still very few mutations characterized thus far.

In my future work I would like to continue characterizing mutations of PRPF3 and PRPF31 and their effect on snRNP formation and splicing.

Acknowledgement

Hereby, I would like to thank all the colleagues from the Dept. of RNA biology at the Institute of Molecular Genetics AS CR for being such a great guys and for creating extraordinarily friendly working environment.

Especially, I would like to thank to our former colleague - Branislav Fleischer, whom I owe the privilege of knowing you, and to David Staněk for all his useful advice and for being such a great boss and tutor.

References

1. Hartong, D.T., E.L. Berson, and T.P. Dryja, *Retinitis pigmentosa.* Lancet, 2006. **368**(9549): p. 1795-809.
2. Hamel, C., *Retinitis pigmentosa.* Orphanet J Rare Dis, 2006. **1**: p. 40.
3. *Retinitis pigmentosa. A symposium on terminology and methods of examination.* Ophthalmology, 1983. **90**(2): p. 126-31.
4. Boughman, J.A., M. Vernon, and K.A. Shaver, *Usher syndrome: definition and estimate of prevalence from two high-risk populations.* J Chronic Dis, 1983. **36**(8): p. 595-603.
5. Beales, P.L., et al., *New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey.* J Med Genet, 1999. **36**(6): p. 437-46.
6. Cohen, M.M., W.H. Knobloch, and R.J. Gorlin, *A dominantly inherited syndrome of hyaloideoretinal degeneration, cleft palate and maxillary hypoplasia (Cervenka syndrome).* Birth Defects Orig Artic Ser, 1971. **7**(7): p. 83-6.
7. Kolehmainen, J., et al., *Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport.* Am J Hum Genet, 2003. **72**(6): p. 1359-69.
8. Hims, M.M., S.P. Diager, and C.F. Inglehearn, *Retinitis pigmentosa: genes, proteins and prospects.* Dev Ophthalmol, 2003. **37**: p. 109-25.
9. Daiger, S.P., *Identifying retinal disease genes: how far have we come, how far do we have to go?* Novartis Found Symp, 2004. **255**: p. 17-27; discussion 27-36, 177-8.
10. Dryja, T.P., et al., *Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa.* Invest Ophthalmol Vis Sci, 1997. **38**(10): p. 1972-82.
11. Berson, E.L., et al., *Vitamin A supplementation for retinitis pigmentosa.* Arch Ophthalmol, 1993. **111**(11): p. 1456-9.
12. Zhao, X., et al., *Derivation of neurons with functional properties from adult limbal epithelium: implications in autologous cell therapy for photoreceptor degeneration.* Stem Cells, 2008. **26**(4): p. 939-49.
13. Sieving, P.A., et al., *Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants.* Proc Natl Acad Sci U S A, 2006. **103**(10): p. 3896-901.

14. Ohguro, H., *[New drug therapy for retinal degeneration].* Nippon Ganka Gakkai Zasshi, 2008. **112**(1): p. 7-21.

15. Viola, M.V. and A.A. Patrinos, *A neuroprosthesis for restoring sight.* Acta Neurochir Suppl, 2007. **97**(Pt 2): p. 481-6.

16. Ohno, M., H. Sakamoto, and Y. Shimura, *Preferential excision of the 5' proximal intron from mRNA precursors with two introns as mediated by the cap structure.* Proc Natl Acad Sci U S A, 1987. **84**(15): p. 5187-91.

17. Valadkhan, S., *The spliceosome: a ribozyme at heart?* Biol Chem, 2007. **388**(7): p. 693-7.

18. Jurica, M.S. and M.J. Moore, *Pre-mRNA splicing: awash in a sea of proteins.* Mol Cell, 2003. **12**(1): p. 5-14.

19. Staley, J.P. and C. Guthrie, *Mechanical devices of the spliceosome: motors, clocks, springs, and things.* Cell, 1998. **92**(3): p. 315-26.

20. Nott, A., H. Le Hir, and M.J. Moore, *Splicing enhances translation in mammalian cells: an additional function of the exon junction complex.* Genes Dev, 2004. **18**(2): p. 210-22.

21. Neugebauer, K.M., *On the importance of being co-transcriptional.* J Cell Sci, 2002. **115**(Pt 20): p. 3865-71.

22. Burge, C. B., Tuschl, T. H. and Sharp, P. A. (1999) "Splicing of precursors to mRNAs by the spliceosomes". In Gesteland, R. F., Cech, T. and Atkins, J. F., eds. "The RNA World", 2nd Ed., Cold Spring Harbor Laboratory Press, Plainview, NY, pp. 525-560.

23. Berget, S.M., *Exon recognition in vertebrate splicing.* J Biol Chem, 1995. **270**(6): p. 2411-4.

24. Hertel, K.J., *Combinatorial control of exon recognition.* J Biol Chem, 2008. **283**(3): p. 1211-5.

25. Dreyfuss, G., et al., *hnRNP proteins and the biogenesis of mRNA.* Annu Rev Biochem, 1993. **62**: p. 289-321.

26. Schellenberg, M.J., D.B. Ritchie, and A.M. Macmillan, *Pre-mRNA splicing: a complex picture in higher definition.* Trends Biochem Sci, 2008. **33**(6): p. 243-6.

27. Guth, S., et al., *Evidence for substrate-specific requirement of the splicing factor U2AF(35) and for its function after polypyrimidine tract recognition by U2AF(65).* Mol Cell Biol, 1999. **19**(12): p. 8263-71.

28. Wu, S., et al., *Functional recognition of the 3' splice site AG by the splicing factor U2AF35.* Nature, 1999. **402**(6763): p. 832-5.

29. Hertel, K.J. and T. Maniatis, *Serine-arginine (SR)-rich splicing factors have an exon-independent function in pre-mRNA splicing.* Proc Natl Acad Sci U S A, 1999. **96**(6): p. 2651-5.

30. Kramer, A., *The structure and function of proteins involved in mammalian pre-mRNA splicing.* Annu Rev Biochem, 1996. **65**: p. 367-409.

31. Chu, V.T., et al., *More than one way to splice an RNA: branching without a bulge and splicing without branching in group II introns.* RNA, 1998. **4**(10): p. 1186-202.

32. Nilsen, T.W., *The spliceosome: no assembly required?* Mol Cell, 2002. **9**(1): p. 8-9.

33. Brow, D.A., *Allosteric cascade of spliceosome activation.* Annu Rev Genet, 2002. **36**: p. 333-60.

34. Bell, M., et al., *p110, a novel human U6 snRNP protein and U4/U6 snRNP recycling factor.* EMBO J, 2002. **21**(11): p. 2724-35.

35. Stanek, D., et al., *Targeting of U4/U6 small nuclear RNP assembly factor SART3/p110 to Cajal bodies.* J Cell Biol, 2003. **160**(4): p. 505-16.

36. Licht, K., et al., *3'-cyclic phosphorylation of U6 snRNA leads to recruitment of recycling factor p110 through LSm proteins.* RNA, 2008.

37. Cioce, M. and A.I. Lamond, *Cajal bodies: a long history of discovery.* Annu Rev Cell Dev Biol, 2005. **21**: p. 105-31.

38. Hall, L.L., et al., *Molecular anatomy of a speckle.* Anat Rec A Discov Mol Cell Evol Biol, 2006. **288**(7): p. 664-75.

39. Rino, J., et al., *A stochastic view of spliceosome assembly and recycling in the nucleus.* PLoS Comput Biol, 2007. **3**(10): p. 2019-31.

40. Kampa, D., et al., *Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22.* Genome Res, 2004. **14**(3): p. 331-42.

41. Smith, C.W. and J. Valcarcel, *Alternative pre-mRNA splicing: the logic of combinatorial control.* Trends Biochem Sci, 2000. **25**(8): p. 381-8.

42. Ben-Dov, C., et al., *Genome-wide analysis of alternative pre-mRNA splicing.* J Biol Chem, 2008. **283**(3): p. 1229-33.

43. Wang, G.S. and T.A. Cooper, *Splicing in disease: disruption of the splicing code and the decoding machinery.* Nat Rev Genet, 2007. **8**(10): p. 749-61.

44. Lopez-Bigas, N., et al., *Are splicing mutations the most frequent cause of hereditary disease?* FEBS Lett, 2005. **579**(9): p. 1900-3.

45. Jacobson, S.G., et al., *Phenotypes of stop codon and splice site rhodopsin mutations causing retinitis pigmentosa.* Invest Ophthalmol Vis Sci, 1994. **35**(5): p. 2521-34.

46. Dry, K.L., et al., *Identification of a 5' splice site mutation in the RPGR gene in a family with X-linked retinitis pigmentosa (RP3).* Hum Mutat, 1999. **13**(2): p. 141-5.

47. Piriev, N.I., J.M. Shih, and D.B. Farber, *Defective RNA splicing resulting from a mutation in the cyclic guanosine monophosphate-phosphodiesterase beta-subunit gene.* Invest Ophthalmol Vis Sci, 1998. **39**(3): p. 463-70.

48. Sullivan, L.S., et al., *Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families.* Invest Ophthalmol Vis Sci, 2006. **47**(7): p. 3052-64.

49. Schmidt-Kastner, R., et al., *Hypoxia-regulated components of the U4/U6.U5 tri-small nuclear riboprotein complex: possible role in autosomal dominant retinitis pigmentosa.* Mol Vis, 2008. **14**: p. 125-35.

50. Gupta, M., P.T. Mungai, and E. Goldwasser, *A new transacting factor that modulates hypoxia-induced expression of the erythropoietin gene.* Blood, 2000. **96**(2): p. 491-7.

51. Grimm, C., et al., *HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration.* Nat Med, 2002. **8**(7): p. 718-24.

52. Keen, T.J., et al., *Mutations in a protein target of the Pim-1 kinase associated with the RP9 form of autosomal dominant retinitis pigmentosa.* Eur J Hum Genet, 2002. **10**(4): p. 245-9.

53. Maita, H., et al., *PAP-1, the mutated gene underlying the RP9 form of dominant retinitis pigmentosa, is a splicing factor.* Exp Cell Res, 2004. **300**(2): p. 283-96.

54. Shirogane, T., et al., *Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and antiapoptosis.* Immunity, 1999. **11**(6): p. 709-19.

55. Maita, H., et al., *Association of PAP-1 and Prp3p, the products of causative genes of dominant retinitis pigmentosa, in the tri-snRNP complex.* Exp Cell Res, 2005. **302**(1): p. 61-8.

56. Xu, S.Y., et al., *A ninth locus (RP18) for autosomal dominant retinitis pigmentosa maps in the pericentromeric region of chromosome 1.* Hum Mol Genet, 1996. **5**(8): p. 1193-7.

57. Horowitz, D.S., R. Kobayashi, and A.R. Krainer, *A new cyclophilin and the human homologues of yeast Prp3 and Prp4 form a complex associated with U4/U6 snRNPs.* RNA, 1997. **3**(12): p. 1374-87.

58. Teigelkamp, S., et al., *The 20kD protein of human [U4/U6.U5] tri-snRNPs is a novel cyclophilin that forms a complex with the U4/U6-specific 60kD and 90kD proteins.* RNA, 1998. **4**(2): p. 127-41.

59. Liu, S., et al., *The network of protein-protein interactions within the human U4/U6.U5 tri-snRNP.* RNA, 2006. **12**(7): p. 1418-30.

60. Little, J.T. and M.S. Jurica, *Splicing factor SPF30 bridges an interaction between the prespliceosome protein U2AF35 and tri-small nuclear ribonucleoprotein protein hPrp3.* J Biol Chem, 2008. **283**(13): p. 8145-52.

61. Boehringer, D., et al., *Three-dimensional structure of a pre-catalytic human spliceosomal complex B.* Nat Struct Mol Biol, 2004. **11**(5): p. 463-8.

62. Lehnert, S., et al., *Protein kinase CK2 interacts with the splicing factor hPrp3p.* Oncogene, 2008. **27**(17): p. 2390-400.

63. Payne, J.M., P.J. Laybourn, and M.E. Dahmus, *The transition of RNA polymerase II from initiation to elongation is associated with phosphorylation of the carboxyl-terminal domain of subunit IIa.* J Biol Chem, 1989. **264**(33): p. 19621-9.

64. Schneider, E., et al., *The cyclin H/cdk7/Mat1 kinase activity is regulated by CK2 phosphorylation of cyclin H.* Oncogene, 2002. **21**(33): p. 5031-7.

65. Wang, A., et al., *Identification and characterization of human genes encoding Hprp3p and Hprp4p, interacting components of the spliceosome.* Hum Mol Genet, 1997. **6**(12): p. 2117-26.

66. Chakarova, C.F., et al., *Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa.* Hum Mol Genet, 2002. **11**(1): p. 87-92.

67. Gamundi, M.J., et al., *Transcriptional expression of cis-acting and trans-acting splicing mutations cause autosomal dominant retinitis pigmentosa.* Hum Mutat, 2008. **29**(6): p. 869-78.

68. Gonzalez-Santos, J.M., et al., *Mutation in the splicing factor Hprp3p linked to retinitis pigmentosa impairs interactions within the U4/U6 snRNP complex.* Hum Mol Genet, 2008. **17**(2): p. 225-39.

69. Comitato, A., et al., *Mutations in splicing factor PRPF3, causing retinal degeneration, form detrimental aggregates in photoreceptor cells.* Hum Mol Genet, 2007. **16**(14): p. 1699-707.

70. Graziotto, J.J., et al., *Decreased Levels of the RNA Splicing Factor Prpf3 in Mice and Zebrafish Do Not Cause Photoreceptor Degeneration.* Invest Ophthalmol Vis Sci, 2008.

71. Greenberg, J., et al., *A new locus for autosomal dominant retinitis pigmentosa on the short arm of chromosome 17.* Hum Mol Genet, 1994. **3**(6): p. 915-8.

72. Siatecka, M., J.L. Reyes, and M.M. Konarska, *Functional interactions of Prp8 with both splice sites at the spliceosomal catalytic center.* Genes Dev, 1999. **13**(15): p. 1983-93.

73. Grainger, R.J. and J.D. Beggs, *Prp8 protein: at the heart of the spliceosome.* RNA, 2005. **11**(5): p. 533-57.

74. McKie, A.B., et al., *Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13).* Hum Mol Genet, 2001. **10**(15): p. 1555-62.

75. Martinez-Gimeno, M., et al., *Mutations in the pre-mRNA splicing-factor genes PRPF3, PRPF8, and PRPF31 in Spanish families with autosomal dominant retinitis pigmentosa.* Invest Ophthalmol Vis Sci, 2003. **44**(5): p. 2171-7.

76. Pena, V., et al., *Structure of a multipartite protein-protein interaction domain in splicing factor prp8 and its link to retinitis pigmentosa.* Mol Cell, 2007. **25**(4): p. 615-24.

77. Small, E.C., et al., *The EF-G-like GTPase Snu114p regulates spliceosome dynamics mediated by Brr2p, a DExD/H box ATPase.* Mol Cell, 2006. **23**(3): p. 389-99.

78. Boon, K.L., et al., *prp8 mutations that cause human retinitis pigmentosa lead to a U5 snRNP maturation defect in yeast.* Nat Struct Mol Biol, 2007. **14**(11): p. 1077-83.

79. al-Maghtheh, M., et al., *Identification of a sixth locus for autosomal dominant retinitis pigmentosa on chromosome 19.* Hum Mol Genet, 1994. **3**(2): p. 351-4.

80. Makarova, O.V., et al., *Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6*U5 tri-snRNP formation and pre-mRNA splicing.* EMBO J, 2002. **21**(5): p. 1148-57.

81. Liu, S., et al., *Binding of the human Prp31 Nop domain to a composite RNA-protein platform in U4 snRNP.* Science, 2007. **316**(5821): p. 115-20.

82. Nottrott, S., H. Urlaub, and R. Luhrmann, *Hierarchical, clustered protein interactions with U4/U6 snRNA: a biochemical role for U4/U6 proteins.* EMBO J, 2002. **21**(20): p. 5527-38.

83. Mordes, D., et al., *Identification of photoreceptor genes affected by PRPF31 mutations associated with autosomal dominant retinitis pigmentosa.* Neurobiol Dis, 2007. **26**(2): p. 291-300.

84. Yuan, L., et al., *Mutations in PRPF31 inhibit pre-mRNA splicing of rhodopsin gene and cause apoptosis of retinal cells.* J Neurosci, 2005. **25**(3): p. 748-57.

85. Rio Frio, T., et al., *Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay.* J Clin Invest, 2008. **118**(4): p. 1519-31.

86. Chang, Y.F., J.S. Imam, and M.F. Wilkinson, *The nonsense-mediated decay RNA surveillance pathway.* Annu Rev Biochem, 2007. **76**: p. 51-74.

87. Schaffert, N., et al., *RNAi knockdown of hPrp31 leads to an accumulation of U4/U6 di-snRNPs in Cajal bodies.* EMBO J, 2004. **23**(15): p. 3000-9.

88. Rivolta, C., et al., *Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations.* Hum Mutat, 2006. **27**(7): p. 644-53.

89. Deery, E.C., et al., *Disease mechanism for retinitis pigmentosa (RP11) caused by mutations in the splicing factor gene PRPF31.* Hum Mol Genet, 2002. **11**(25): p. 3209-19.

90. Wilkie, S.E., et al., *Disease mechanism for retinitis pigmentosa (RP11) caused by missense mutations in the splicing factor gene PRPF31.* Mol Vis, 2008. **14**: p. 683-90.

91. Santama, N., C.G. Dotti, and A.I. Lamond, *Neuronal differentiation in the rat hippocampus involves a stage-specific reorganization of subnuclear structure both in vivo and in vitro.* Eur J Neurosci, 1996. **8**(5): p. 892-905.