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Review

mRNA metabolism and neuronal disease

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ABSTRACT

To serve as templates for translation eukaryotic mRNAs undergo an elaborate processing and maturation pathway. In eukaryotes this process comprises the synthesis of mRNA precursors, their processing and transport to the site of translation and eventually their decay. During the entire life cycle, mRNAs interact with distinct sets of *trans*-acting factors that determine their fate at any given phase of gene expression. Recent studies have shown that mutations in components acting in *trans* on mRNAs are frequent causes of a large variety of different human disorders. The etiology of most of these diseases is, however, only poorly understood, mostly because the consequences for mRNA-metabolism are unclear. Here we discuss three prominent genetic diseases that fall into this category, namely spinal muscular atrophy (SMA), retinitis pigmentosa (RP) and X-linked syndromic mental retardation (XLMR). Whereas SMA and RP can be directly linked to mRNA processing, XLMR results from mutations in the mRNA surveillance system. We discuss how defects in mRNA maturation and turnover might lead to the tissue specific defects seen in these diseases.

1. Introduction

The maturation of protein coding messenger RNA (mRNA) and its commitment to translation are key events in gene expression. While mRNA was long considered a rather passive carrier of information, it became increasingly clear in recent years that it is also central to a variety of post-transcriptional gene regulatory mechanisms. These mechanisms affect almost all steps in the life cycle of an mRNA and involve the formation, modification and degradation of mRNA-protein complexes (mRNPs) [1]. The starting point of mRNP production in eukaryotes is the generation of a precursor mRNA (pre-mRNA) by RNA polymerase II (RNAP II), which is then processed to reach maturity. Processing events include capping of the 5′ end [2], typically pre-mRNA splicing to remove non-coding introns [3], and the generation of a 3′ end by cleavage and polyadenylation [4]. Proper maturation of mRNA in the nucleus is a prerequisite for its subsequent export to the cytosol and determines its subcellular localization as well as its ability to associate with the translation machinery. In the course of this pathway, the mRNA attracts and ejects the *trans*-acting factors that make up the mRNP and are crucial for the respective phase (Fig. 1).

The initial association of proteins with mRNAs occurs as soon as the 5′-end of a nascent transcript emerges from RNAP II (Fig. 1). Many of the factors that are connected to its processing and the subsequent nuclear export associate with the mRNA in this initial phase [5]. These include the capping and polyadenylation machinery, the spliceosome and cleavage and polyadenylation factors, all of which are co-transcriptionally recruited onto mRNA [6]. As part of nuclear processing, not only the RNA-structure is altered but also its association with proteins [5]. This process, which is referred to as mRNP remodeling, licenses the mRNP for nuclear export [7]. Important marks of this licensing process are the splicing-dependent deposition of exon junction complexes (EJCs) and the recruitment of mRNA export factors such as the metazoan transcription-export (TREX) complex [8,9]. Remodeling continues in the cytosol where some mRNAs associate with transport factors that establish the subcellular localization that is required for their functionality [10]. Translation of mRNPs by ribosomes leads to further remodeling, for example the replacement of the nuclear cap binding complex by eIF4E and the disassembly of the EJCs [11,12].

As already implied from the above said, mRNAs interact at all stages of their life with distinct sets of *trans*-acting factors (mostly proteins but also small RNAs, RNPs and metabolites) to form mRNPs. This "mRNP code" determines the fate of any given mRNA and is thus the major denominator of processing and post-transcriptional gene regulation. Perhaps not surprisingly,

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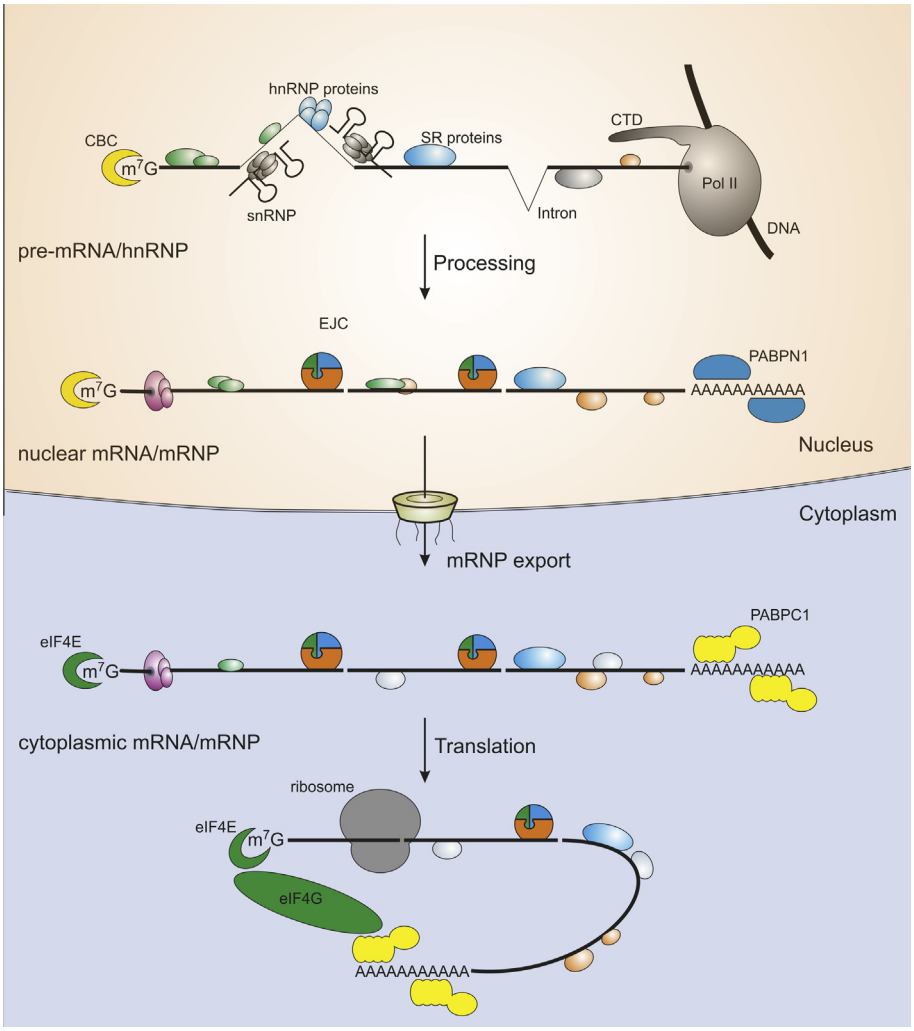

Fig. 1. Assembly and remodeling of mRNPs in the nucleus and cytoplasm. An initial set of RNA-binding proteins associates with the mRNA during transcription by RNA polymerase II. Early mRNP components include hnRNP proteins and the mRNA processing machinery, for example the snRNPs of the spliceosome. The composition of the mRNP is altered during different gene expression steps and actively influences its fate. After transport to the cytoplasm the mRNA is translated and eventually degraded. Important components of the mRNP are depicted.

therefore, disruptions of the proper mRNP code by mutations in RNA-binding proteins, their mRNA binding sites or their recruitment and remodeling factors are increasingly recognized as causes of human disease. This includes a variety of non-neurological disorders like oculopharyngeal muscular dystrophy [13], hypotrichosis simplex [14], and various forms of cancer but is especially prominent in the etiology of neuronal disease. Examples include amyotrophic lateral sclerosis (ALS), which can be caused by defects in RNA-binding proteins [15] as well as toxic RNA trinucleotide repeat expansions [16,17], Fragile X Syndrome (FXS), which is caused by mutations in the FMR1 gene that encodes a key regulator of neuronal mRNA translation [18], and spinocerebellar ataxia which is also often caused by trinucleotide repeat expansions [19]. Here, we focus on diseases that are directly linked to mRNA processing and mRNP remodeling and summarize recent progress that has been made in the understanding of the disorders spinal muscular atrophy (SMA), retinitis pigmentosa (RP) and X-linked syndromic mental retardation (XLMR). These diseases are caused by defects in the assembly of the spliceosomal machinery, the function of the spliceosome itself and the mRNA surveillance pathway, respectively.

2. Spinal muscular atrophy: defects in the formation of the splicing machinery

SMA is a prime example of a disease linked to the general pre-mRNA splicing machinery. The degeneration of α-motoneurons in the spinal cord is the main cellular characteristic of this common autosomal recessive disorder. Compound heterozygous mutations or deletions of the *SMN1* gene copy, which prevent the expression of functional SMN protein, are the sole cause of this disease [20]. Humans contain one or several copies of a second SMN-gene (termed *SMN2*). Even though both genes potentially encode for the same protein, a single nucleotide C to T transition in exon 7 results in mis-splicing of the majority of the *SMN2* encoded pre-mRNA [21–23]. This results in the production of a truncated protein from *SMN2*, which is unstable and non-functional (Fig. 2). SMA therefore results from SMN-deficiency, and the deletion of the *SMN1* gene cannot be fully compensated by *SMN2*. There are no SMA patients known that completely lack SMN and the complete knockout of SMN in various model organisms such as mice, *Caenorhabditis elegans* and *Schizosaccharomyces pombe* is lethal, suggesting that SMN fulfills an essential function [24].

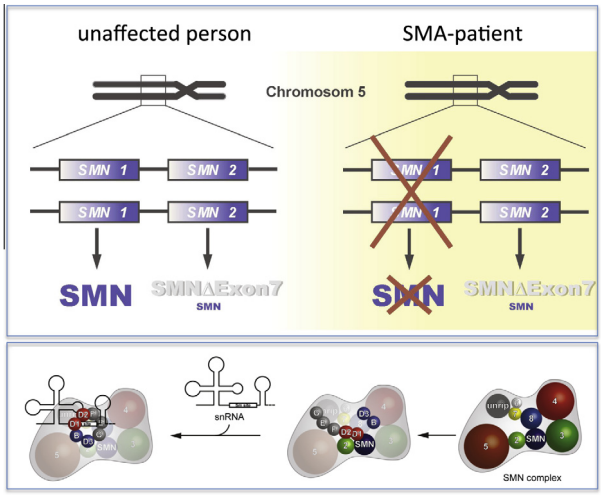

Fig. 2. Spinal Muscular Atrophy is caused by reduced expression of the snRNP assembly factor SMN. (upper panel) Humans have two SMN genes (SMN1 and SMN2). SMN1 produces full-length and functional SMN protein whereas SMN2 produces predominantly a truncated and non-functional version (SMNΔExon7, left). Due to mutations in the SMN1 gene only very low levels of SMN protein are produced in SMA patients (right). (lower panel) The SMN protein is part of an intricate system that ensures the assembly of spliceosomal snRNPs. The SMN complex consists of SMN as well as the proteins Gemins2–8 (denoted 2–8 in the figure) and unrip. The Sm proteins (termed B, D1, D2, D3, E, F and G) are recruited to the SMN complex and loaded onto the snRNA.

The functional analysis of SMN revealed a link to the pre-mRNA processing spliceosome. This dynamic macromolecular complex catalyses the excision of non-coding sequences (introns) from pre-mRNAs thereby generating the open reading frame for translation. The major spliceosome, which splices the vast majority of pre-mRNAs is composed of a group of RNA-protein complexes termed small nuclear ribonucleoproteins (snRNPs) U1, U2, U4/6 and U5 as well as a large number of additional proteins. Cells possess also a "minor" spliceosome, which splices rare non-canonical (minor) introns found in some pre-mRNAs [25]. Even though some components of this machinery differ, their overall biochemical composition is very similar to the major spliceosome and includes homologous U snRNPs (termed U11, U12, U4atac and U6atac snRNPs) and associated splicing factors.

A large body of evidence has implicated SMN in the assembly of the Sm-class of U snRNPs and hence also the formation of the functional major and minor spliceosomes. The Sm-class of U snRNPs are assembled in cells in a complex and segmented biogenesis pathway. This pathway starts with the transcription of the snRNA by polymerase II and its subsequent nuclear export to the cytoplasm. The U snRNA then binds to a set of common (Sm) proteins that form the core RNA of these particles as well as additional specific U snRNP factors. After additional maturation steps, including the hypermethylation of the m^7G-cap to the m_3G-cap the assembled snRNP particle is imported into the nucleus and eventually incorporated into the spliceosome [26–28].

The best-understood role of SMN in the biogenesis cycle is the cytosolic assembly of the common Sm proteins with the snRNA and the subsequent nuclear import and maturation to fully functional particles [29] (Fig. 2). The SMN protein acts in the context of a macromolecular unit, which also contains 7 proteins collectively referred to as "Gemins" as well as the factor unrip. This so-called SMN complex ensures the efficient loading of Sm proteins onto the U snRNP and hence the formation of the RNP core of these particles [30–33]. As this core is important for the subsequent nuclear import and incorporation into spliceosomes, SMN (and the entire SMN-complex) can be considered to be of major importance for the splicing machinery in general.

Whether the disease SMA is a direct consequence of perturbations in the U snRNP biogenesis pathway or downstream events thereof such as splicing is an intriguing albeit still controversially discussed question. What appears undisputed is that SMN deficiency provokes a marked reduction of assembly activity and U snRNP levels in most cells tested so far, including neuronal cells derived from animal models of SMA. Of note, the formation of snRNPs of the minor spliceosomes appears to be more affected than snRNPs of major spliceosomes [34–37]. The downstream consequence of cellular U snRNP shortage is, however, less clear. In agreement with the disturbance of pre-mRNA processing, massive alterations in the splicing pattern of a variety of different mRNAs were observed in animal models such as mice, flies and even yeast. Consistent with a pronounced reduction of the minor snRNPs biogenesis, mRNAs containing minor introns are frequent among the mis-spliced transcripts [37–41]. Whether these alterations directly result from SMN deficiency or whether they are secondary to cell/tissue degeneration needs to be sorted out by further studies that include the analysis of animal models at time points prior to disease onset. It is presently disputed whether these mis-splicing events are causative for the disease [42]. In favor of this view, a mis-spliced transcript containing a minor intron has been identified in a Drosophila melanogaster model of SMA. As the encoded protein, termed Stasimon is required for motor circuit function, this finding provided an explanation for the tissue specificity of the disease [43,44]. On the other hand, the investigation of age-matched Smn-null and Smn flies by a deep sequencing approach failed to detect altered expression of mRNAs containing minor introns [44]. Likewise, the analysis of mice models of SMA gave contradictory results. In a microarray-based study, late symptomatic mice displayed widespread and tissue-specific changes in the splicing pattern of a large number of mRNAs, no matter whether they contained major or minor introns [37]. Furthermore, an RNA deep sequencing approach revealed neuron specific mRNA dysregulations in presymptomatic SMA mice [41]. In contrast, other studies reported that changes in mRNA patterns do not appear in pre-symptomatic SMA mice, suggesting that the observed splicing effects are downstream effects of the loss of SMN expression [38]. Hence, it is currently unclear whether mis-splicing events observed in SMA animal models contribute to the disease phenotype and whether similar events also contribute to the disease in humans.

Apart from SMN's role in RNP biogenesis, the protein has been reported to fulfil additional functions, some of which are neuron specific and related to mRNA metabolism [45–48]. At present, it cannot be excluded that these functions, rather than the above-mentioned role of SMN in splicing contribute to or even cause the disease phenotype. Of potential relevance for the understanding of the SMA etiology is the role of SMN in the axonal transport of certain mRNAs. SMN is present in axons of neurons, including motor neurons affected in SMA. As a hallmark of motor neurons is their very long axons (in humans they can be up to one meter) it is tempting to speculate that reduced SMN levels selectively affect motor neurons because this cell type critically depends on mRNA transport along the axon.

3. Retinitis pigmentosa: defects in the splicing machinery

Whereas the analysis of the etiology of SMA revealed a link to the production of spliceosomal U snRNPs and hence the pre-mRNA processing spliceosome, some forms of the disease retinitis pigmentosa (RP) are characterized by mutations in components of the spliceosome itself. RP is a hereditary eye disease that

displays extreme genetic heterogeneity, with more than 60 underlying disease genes identified so far (RetNet, the Retinal Information Network, www.sph.uth.edu/retnet/). Most protein products of these genes are involved in various aspects of photoreceptor biology, including their development, their structure and phototransduction itself. Despite this variety in disease genes, the phenotype of isolated RP is very specific, affecting only the retina. Therefore, it came as a surprise when it was discovered that a mutation in PRPRF31, a core component of the spliceosome, was found to cause RP [49]. Since then, however, several splicing factors were identified as RP disease genes, including 7 spliceosomal proteins (PRPF31, PRPF16, PRPF8, PRPF6, PRPF4, PRPF3 and SNRNP200) [49–56]. To date, it is estimated that more than 12% of all cases of autosomal dominant RP (adRP) are caused by defects in splicing factors [57].

All RP-linked splicing factors are core components of the spliceosome. The formation of this multimeric complex begins when the U1 snRNP subunit binds to the 5′ splice-site of a pre-mRNA (Fig. 3) (for review, see [25]). With the help of the non-snRNP splicing factor U2AF, the U2 snRNP subunit is then recruited to the branch point located close to the 3′ splice-site of the intron. Next, this so-called "A-complex" is joined by a pre-assembled U4/U6.U5 tri-snRNP to form the B complex, which upon a number of structural and compositional rearrangements gives rise to the catalytically active complexes B* and C which catalyze the first and second transesterification step of the splicing reaction, respectively. During the activation of the spliceosome and after the completion of the splicing reaction, the individual snRNPs are released as monomers, from which they are recycled and then can participate in another round of splicing [58]. While the exact molecular functions of the RP-linked splicing factors during the spliceosomal cycle are only beginning to be uncovered, it was clear since their first discovery in yeast that these factors are crucial for spliceosome formation and intron removal [59]. This creates the paradoxical situation that heterozygous mutations of genes that are considered essential in all cells of the body evoke a phenotype that is restricted to very few or even a single type of cells.

Because a single gene product may have more than one biological activity in the cell ("protein moonlighting"), it is difficult to exclude that an individual disease gene product, although considered a housekeeping gene, might have an additional, cell-type specific function. However, unlike in the case of SMA, the defective splicing factors in RP are all part of the same multimeric subcomplex of the spliceosome, the U4/U6.U5 tri-snRNP (Fig. 3), and it is highly unlikely that the individual components all have an additional photoreceptor-specific function. Therefore, either the tri-snRNP itself has a yet-to-be discovered function in photoreceptors, or the etiology of RP is directly linked to pre-mRNA splicing.

One hypothesis of how defects in pre-mRNA splicing can elicit a cell-type specific defect is that there is a cell-type specific transcript that has a very high demand for splicing activity [60]. As photoreceptors renew 10% of their rhodopsin containing discs in a diurnal circadian rhythm and thus have to synthesize an enormous amount of rhodopsin mRNA [61], the rhodopsin transcript was considered a prime candidate for mediating this effect. Indeed, it was demonstrated in HEK293 cells that splicing factor defects inhibit the splicing of a transfected minigene construct containing the short intron 3 of the human rhodopsin transcript [62]. However, a number of reasons speak against mis-splicing of this individual transcript as the disease-causing event: First, when the full-length rhodopsin transcript was analyzed, no defects in the splicing of this intron were found [63]. Second, splicing factor deficiency causes photoreceptor defects in zebrafish [64], which do not

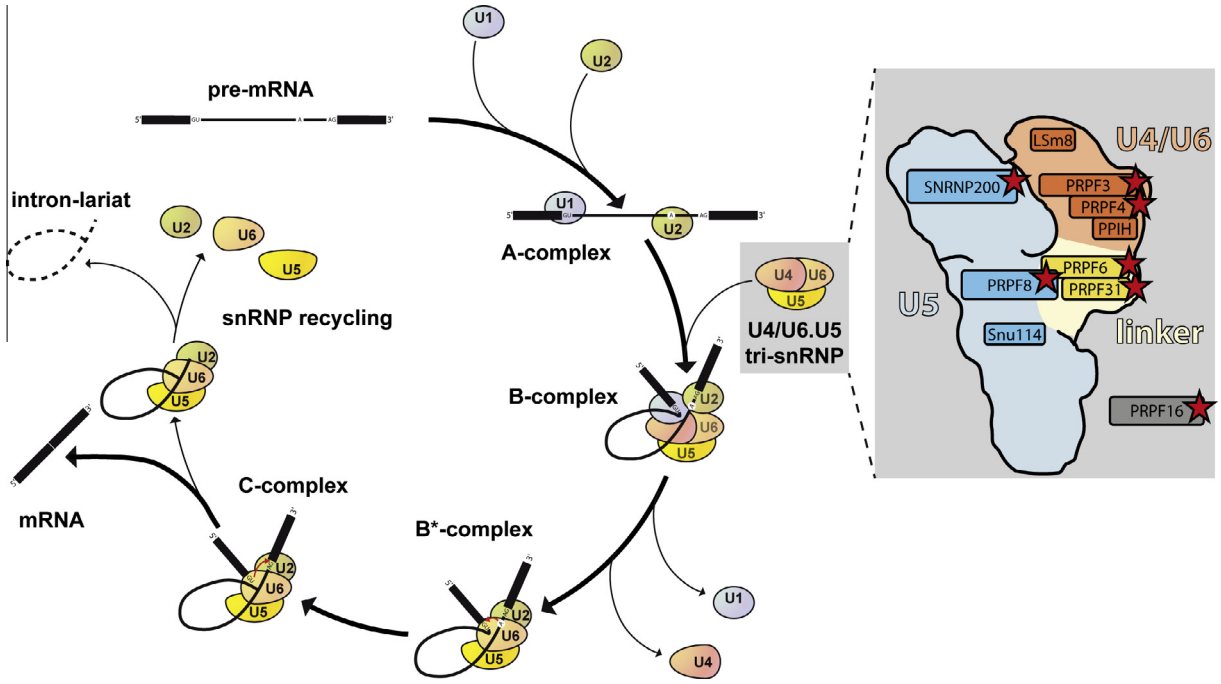

Fig. 3. Spliceosomal proteins and retinitis pigmentosa. (left panel) The spliceosomal cycle assembles the spliceosome on its pre-mRNA substrate. First, the snRNPs U1 and U2 bind to the 5′- and 3′-splice sites of a pre-mRNA, respectively to give rise to the A complex. The tri-snRNP joins this complex to give rise to the B complex. Together with the tri-snRNP, the RP-linked splicing factors enter the spliceosomal cycle. After the sequential loss of the snRNPs U1 and U4, the active C complex is formed and the two transesterification steps of the splicing reaction occur. Of note, the RP-linked splicing factors PRPF3, PRPF4, and PRPF31 are lost upon activation, while PRPF6, PRPF8 and SNRNP200 remain in the C complex (The BΔU1 complex that arises after the loss of the U1 snRNP from the B-complex has been omitted for clarity). (right panel) Schematic of the tri-snRNP and the RP-linked spliceosomal proteins. The position of a subset of tri-snRNP proteins is depicted on a structural model derived from cryo-electron-microscopy [112]. Proteins encoded by RP disease genes are marked with a red star. The RP-linked splicing factor PRPF16 is not part of the tri-snRNP but joins the spliceosome during the formation of the C-complex. Adapted from Häcker et al. [112] with permission from Macmillan Publishers Ltd.

possess introns in the rhodopsin gene [65]. Third, emerging evidence from a mouse model of splicing factor-linked RP suggests that the primary defect lies in the retinal pigment epithelium (RPE) rather than the photoreceptors themselves [66]. While it must be noted that these animals display a late-onset phenotype that might not completely resemble the situation in humans, it is clear that their primary phenotype is in the RPE, which does not express the rhodopsin transcript. Nevertheless, even without rhodopsin being the prime candidate for mediating the detrimental effects of splicing factor mutations, the retina does seem to have an extraordinarily high demand for splicing activity, as it has a 7-fold higher expression level of snRNAs than other tissues [60]. Accordingly, it has been proposed that the vulnerability of retinal cells to splicing factor defects might stem from an unmet requirement for the production of sufficient amounts of mature retinal mRNAs [67].

It is important to note, however, that the shortfall of mature mRNA is only one of the potential mechanisms for eliciting photoreceptor cell death. For example, failure to remove an intron might generate novel protein variants that are toxic for the cell. Interestingly, retention of the rhodopsin intron 3 generates a non-sense transcript that translates into a truncated protein with a very efficient peroxisomal localization sequence at its C-terminus (B. Linder and U. Fischer, unpublished data). Normally, the NMD surveillance pathway eliminates transcripts affected by such premature termination events, but a small portion of the highly expressed rhodopsin transcript might escape this mechanism. Such non-sense transcripts are expected to be generated in large amounts and from a variety of genes if the general splicing machinery is compromised. The effects of non-sense mutations are well documented in human disease. NMD can modify the expressivity of such mutations and mask otherwise dominant phenotypes [68]. One reason for the vulnerability of this tissue to splicing defects might thus be an "overload" of the NMD pathway. Testing whether the retina expresses increased levels of the surveillance machinery to keep up with its high splicing activity will help to gain insight into the extent at which NMD modulates disease expressivity in splicing factor-linked RP.

Another mechanism by which defective tri-snRNPs might influence the fate of the mRNAs produced is the deposition of the exon junction complex on the spliced mRNA. This process is mediated by the tri-snRNP associated protein CWC22 [69,70]. It has been demonstrated that defects in CWC22 can lead to a spliced mRNA that is devoid of EJCs [71,72]. Hence, if CWC22 is not properly recruited to the spliceosome, this might alter the composition of the resulting mRNP. This in turn can lead to a decreased translation efficiency as well as to the evasion of NMD. Therefore, one might envisage that tri-snRNP defects that interfere with CWC22 recruitment could affect the expression of an mRNA without even altering its splicing efficiency or splicing pattern. However, if EJC formation is indeed affected in RP remains to be determined.

Finally, the tri-snRNP is not only important for constitutive splicing, but also plays a role in the regulation of alternative splicing events [73]. Because the joining of the tri-snRNP to pre-mRNA-associated U1 and U2 snRNPs determines which 5′ and 3′ splice sites are used, this can give rise to mRNAs that either include or skip specific exons. Interestingly, it has been found that a number of alternative splicing events are affected in lymphoblasts derived from RP patients [60], suggesting that this might be a key mechanism by which defective tri-snRNPs influence the photoreceptor transcriptome.

Independent of the mechanism that ultimately links splicing factor defects to photoreceptor cell death, it is clear that the tri-snRNP particle plays a special role in the etiology of this disease, as all RP-linked splicing factors reported so far are components of this particle (except PRPF16, which joins independently of the tri-snRNP but directly binds to its components [74]). The fact that the affected proteins are all part of the same multiprotein complex opens up the possibility that defects in different tri-snRNP components might act together to decrease the level of functional tri-snRNPs below the threshold required in retinal cells. Such a model has several implications for the genetics of RP. As mutant alleles of different splicing factors would cause disease only in combination, a large number of unaffected carriers and a high rate of simplex cases is expected, exactly as it is observed for RP [75,76]. It might also explain why in some animal models the heterozygous knockout of a tri-snRNP protein fails to evoke retinal defects [77,78]. Thus a "single hit" might not be sufficient to decrease tri-snRNP function below the pathogenic threshold. Such a mechanism has been reported for other protein complexes involved in retinal degeneration such as the RDS/ROM complex and the Bardet–Biedl-Syndrome (BBS) complex [79]. As a result, in these cases the disease exhibits an oligogenic pattern of inheritance [80]. Therefore, akin to what has been postulated for BBS [81], the relatively large pool of unidentified RP disease genes is likely to contain additional tri-snRNP splicing factors. The identification of novel RP mutations in splicing factors will hopefully not only yield insights into the dynamic mechanisms of pre-mRNA splicing, but also offer strategies for novel therapeutic approaches in RP.

4. X-linked syndromic mental retardation caused by mutations in the UPF3b gene: regulation of brain development and function by mRNA quality control

Whereas the diseases SMA and RP can be linked to pre-mRNA processing, the third neuronal disease we want to discuss here provides a link to the mRNA quality control pathway termed nonsense-mediated mRNA decay (NMD). This mRNA surveillance mechanism ensures that transcripts containing premature translation termination codons (PTCs) are eliminated in eukaryotic cells [82]. Thereby, NMD suppresses the synthesis of C-terminally truncated proteins from mutated mRNAs and also regulates the expression of many genes, which represent endogenous NMD targets [83]. Mechanistically, the presence of a PTC is detected during translation termination. Termination at an inappropriate position, as determined by either architecture of the mRNA or mRNP composition, leads to the assembly of a protein complex, consisting of the proteins UPF1, UPF2 and UPF3 (Fig. 4A). This so-called surveillance complex coordinates the recruitment of nucleases and decapping complexes to the substrate mRNA in order to initiate its degradation [84]. The three UPF proteins are conserved in all eukaryotic organisms and act as central players of the NMD process [85]. UPF3 interacts with the NMD factor UPF2 and with the exon junction complex (EJC), a set of NMD-promoting factors, which is assembled in the proximity of spliced-out introns on mRNAs [86,87]. UPF2 functions as an adaptor protein that directly binds to both UPF1 and UPF3 [85]. UPF1, an ATP-dependent RNA helicase, interacts with mRNAs at a position downstream of the termination codon and is regulated by its binding partner UPF2 [88].

In humans, UPF3 is expressed from two paralogous genes, UPF3A and UPF3B, which share approximately 60% amino acid identity and display a comparable domain architecture [89]. UPF3A and UPF3B are located on the long arm of chromosome 13 and on the long arm of chromosome X, respectively. While the absence of UPF1 and UPF2 leads to an early embryonic lethality in mice [90,91], the physiological role of the two mammalian UPF3 genes is not well understood. Although both UPF3 proteins are considered to serve redundant functions, functional differences between them have been reported. UPF3B seems to be more active

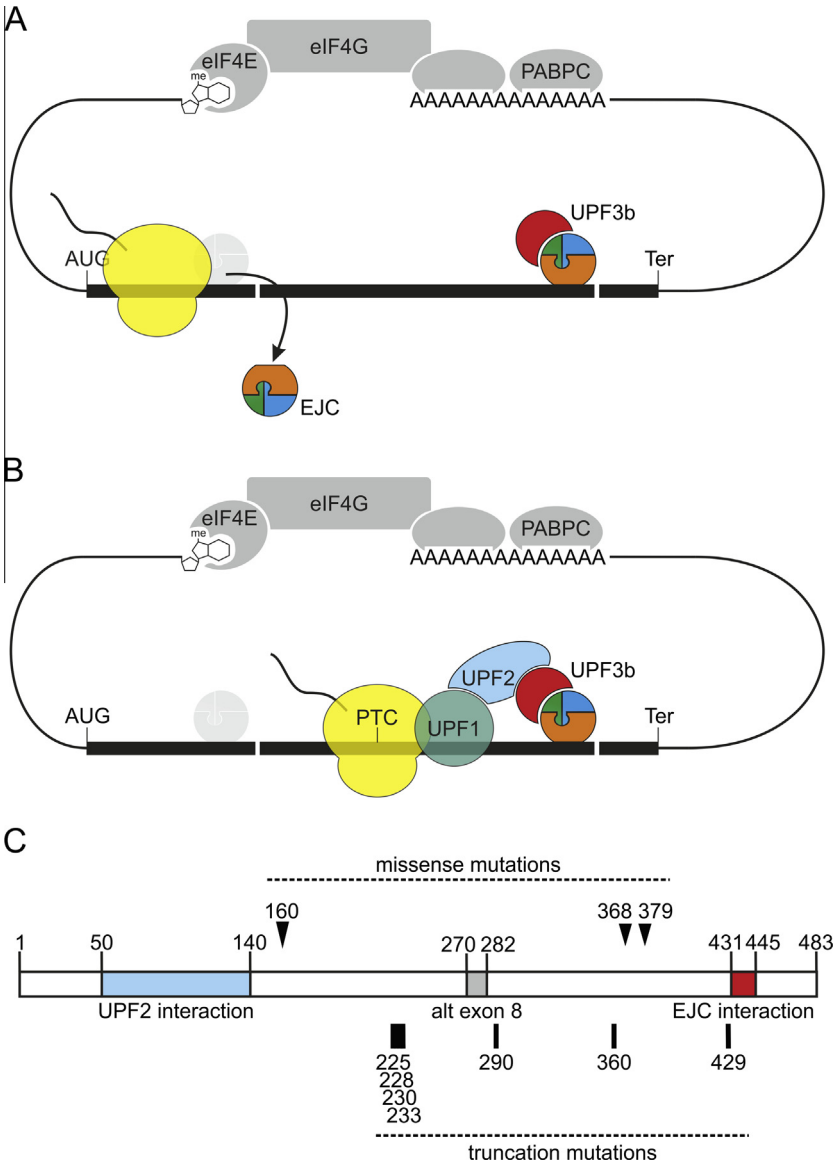

Fig. 4. Simplified model of mammalian nonsense-mediated decay. Exon junction complexes (EJCs) are assembled upstream of splice junction in the nucleus and remain bound to their binding site until the mRNA is translated in the cytoplasm. UPF3b interacts directly with core proteins of the EJC. (A) Transcripts with a normal termination codon are translated into full-length proteins. During translation, EJCs are removed from the mRNA by ribosomes. (B) A premature termination codon (PTC) elicits NMD when at least one EJC is located downstream. Translating ribosomes stop at the termination codon and a surveillance complex consisting of UPF1, UPF2 and UPF3 supports the recognition of the EJC. Eventually, decay factors are recruited by UPF1 to degrade the substrate mRNA (not shown). (C) Schematic domain architecture of UPF3b. Domains interacting with UPF2 and the EJC are depicted. Positions of disease-associated missense and nonsense/frameshift mutations are shown above and below the scheme, respectively.

in triggering NMD and translation regulation and to bind more efficiently to UPF2 than UPF3A [92,93]. Since the interaction with UPF2 stabilizes the UPF3A protein, the presence of UPF3B destabilizes UPF3A [93].

Mutations in the UPF3B gene have been identified in patients with neuro-developmental disorders and intellectual disability (ID), indicating a connection between the NMD pathway and X-linked mental retardation (XLMR) [94]. Ten families carrying UPF3B mutations have been analyzed and seven truncation mutations and three missense mutations in the UPF3B gene have been identified (Fig. 4B) [94–98]. It is very likely that truncation mutations as well as missense mutations disturb UPF3B protein function, although they are located in different regions of UPF3B (Fig. 4B). A broad range of clinical symptoms including autism, schizophrenia and an abnormal long and thin face have been observed in patients with UPF3B mutations [94]. This suggests that UPF3B deficiency can lead to a variety of disease manifestations and psychiatric disorders [95]. There was no consistent diagnosis in patients with UPF3b mutations apart from ID. Therefore, highly variable clinical expressivity of identical mutations has been suggested [94–98]. Although the underlying mechanism for the clinical variability is not known, the phenotype of patients with UPF3B mutations is likely modulated by the functional redundancy of UPF3B and UPF3A. Increased levels of UPF3A protein were observed in patients' cell lines, suggesting that UPF3A partially rescues NMD in the absence of functional UPF3B [93,99]. Moreover, UPF3A levels inversely correlated with the severity of the neurological symptoms and may explain inter-individual differences between patients from the same family [94,99]. Hence, UPF3A is a potential modifier of the clinical phenotype of UPF3B patients, but the regulation of UPF3A protein levels remains elusive.

Recently, an association of heterozygous deletions of a genomic region that include UPF2 with neuro-developmental disorders has been reported [100]. In addition, a de novo missense mutation in

UPF2 has been identified in a patient with schizophrenia [101]. Similarly, copy number losses of genomic regions encompassing UPF3A and copy number gains in genomic regions encompassing genes involved in NMD were frequently found in patients with ID [100]. Specifically, copy number gains of the NMD-specific endonuclease SMG6 and two EJC factors, EIF4A3 and RNPS1, have been described [100]. Patients with such copy number variations of other NMD genes show a wide range of clinical symptoms, similar to patients with UPF3B mutations. This supports the idea that misregulation of NMD predisposes for a wide range of neuro-developmental and psychiatric disorders [102,103].

How does NMD and in particular UPF3B contribute to normal brain development and neuronal and synaptic homeostasis? This question is very difficult to answer and several different mechanisms have been suggested. For example, the eIF4A3 protein, a component of the NMD-activating EJC, has been shown to associate with neuronal mRNA granules and dendritic mRNAs [104]. Furthermore, the central NMD factor UPF1 has been identified in multi-protein complexes isolated from human postsynaptic density isolated from the neocortex [105]. Thus, NMD may regulate the levels of mRNAs, which contribute to establish and maintain synaptic functions.

One potential brain-specific substrate of NMD is the PTBP2 mRNA, which encodes a global regulator of neuronal-specific splicing programs [106,107]. The splicing of PTBP2 is regulated by its homolog PTBP1 in non-neuronal cells and neural progenitor cells. In these cells the presence of PTBP1 leads to skipping of exon 10 of PTBP2. This isoform of PTBP2 contains a frameshift, which leads to its degradation by NMD. However, when neural progenitor cells differentiate into immature neurons, the expression levels of PTBP1 strongly decrease. This results in inclusion of exon 10 into the PTBP2 mRNA and the biosynthesis of functional PTBP2 protein, which controls splicing events involved in the initiation of neuronal maturation [108]. Another neuron-specific NMD substrate is the Arc mRNA, which is one of the most abundant transcripts in dendrites and up-regulated upon synaptic activation. Arc protein is required for maintenance of long-term potentiation. The down-regulation of eIF4A3 in cultured neurons leads to an increased abundance of the Arc protein, which in turn increased excitatory synaptic strength [104]. Since this observation is in conflict with data showing that the genetic knockout of Arc increase both synaptic strength and Arc overexpression in cultured hippocampal neurons led to a defect in synaptic scaling [109] future work will be required to solve this apparent discrepancy.

The important function of NMD factors during brain development is underscored by their strict regulation. Notably, neuron-specific regulation of NMD is mediated by miR-128, which targets UPF1 and the EJC component MLN51. While undifferentiated P19 cells express only small amounts of miR-128, they dramatically upregulate mature miR-128 transcripts to repress NMD when differentiation into neuron-like cells is induced [110]. Furthermore, the expression of several NMD factors is regulated by a negative feedback loop, which has been suggested to fine-tune the activity of NMD in a cell type-dependent manner [111].

Based on the findings summarized above it is likely that NMD plays an important role in the central nervous system. Therefore, mutations and copy number variations that interfere with NMD activity and alter neuronal gene expression contribute to human neurodevelopmental disorders.

5. Conclusions and perspectives

The above diseases represent just a small selection of a growing number of disorders that are caused by mutations in factors that act on, or associate with, mRNA. An interesting common aspect is that the underlying genetic defects appear to affect factors involved primarily in housekeeping, rather than cell type specific functions. This is probably best underlined in the case of RP, where several components of the tri-snRNP are affected, thereby making it unlikely that the tissue-specific phenotype is caused by additional, cell-type specific functions of the mutant proteins. However, also in the case of SMA and XLMR there is evidence that it is the defective housekeeping function of the affected protein that causes the neuron-specific phenotype rather than an additional, cell-type specific function: For mutations in SMN, it has been shown that the end-product of the snRNP biogenesis pathway can rescue the neuronal phenotype in zebrafish [34]. In the case of NMD, it was found that – similar to the situation found in RP – several components of this pathway are linked to neuronal disease. In addition, while the main function of NMD is the surveillance of the transcriptome for erratic PTCs, it has been demonstrated that the same decay pathway is responsible for the post-transcriptional regulation of important neuronal mRNAs, so that it is unlikely that an additional, non-NMD function of these proteins is responsible for the neuron-specific phenotype. It should be mentioned, however, that at present for none of the disease-related factors discussed here, a tissue specific activity, in addition to their housekeeping functions can be excluded that contributes to the etiology.

Why specific cells are especially vulnerable to defects in general mRNA metabolism is therefore a key question that concerns the etiology of SMA, RP and XLMR alike. One explanation for this paradoxical situation might be that the affected pathways in SMA, RP and X-linked syndromic mental retardation ensure the catalytic conversion of a large variety of different (mRNA) substrates by splicing and NMD, respectively. Considering that there are "good" substrates (i.e. those that are efficiently recognized and converted) and "weak" ones, it is tempting to speculate that mutations in general mRNA processing factors do not affect all substrates alike. Such a scenario could indeed present a general explanation for how defects in housekeeping pathways convert into a tissue specific phenotype. The systematic analysis of mRNAs by current high-throughput technologies should enable the identification of the affected transcripts and allow detailed insight into the etiologies of this type of diseases.

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