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REVIEW

Variant snRNPs: New players within the spliceosome system

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ABSTRACT

Much evidence is now accumulating that, in addition to their general role in splicing, the components of the core splicing machinery have extensive regulatory potential. In particular, recent evidence has demonstrated that de-regulation of these factors cause the highest extent of alternative splicing changes compared to de-regulation of the classical splicing regulators. This lack of a general inhibition of splicing resonates the differential splicing effects observed in different disease pathologies associated with specific mutations targeting core spliceosomal components. In this review we will summarize what is currently known regarding the involvement of core spliceosomal U-snRNP complexes in perturbed tissue development and human diseases and argue for the existence of a compensatory mechanism enabling cells to cope with drastic perturbations in core splicing components. This system maintains the correct balance of spliceosomal snRNPs through differential expression of variant (v)U-snRNPs.

Overview of the splicing machinery

Alternative splicing is the principal mechanism by which our cells remodel the transcriptome to generate extensive opportunities for gene regulation, which are required to drive cell differentiation and development.[1,2] Constitutively expressed uridyl-rich small nuclear (U-sn)RNAs, in the form of ribonucleoproteins (U-snRNPs), are well known for their pivotal role in mRNA splicing events by dictating precisely where splicing occurs.[3-6] The vast majority of introns (99%) are processed by the U2-type spliceosome consisting of five snRNAs, U1, U2, U4, U5 and U6, and hundreds of associated proteins. A minor class of introns (< 849) is excised by the U12-type spliceosome, which is analogous to the major complex but contains four unique snRNAs, including U11, U12, U4atac and U6atac, and several other unique proteins.[7] These core components of the spliceosome use molecular mechanisms principally involving snRNA: pre-mRNA base-pairing interactions to target intronic sequences for excision. Hundreds of additional proteins, known as splicing factors, play important roles in remodeling the composition of these snRNP subcomplexes, at particular stages throughout the splicing process, ensuring the fidelity and specificity of the process.[8-10] However, the choice of which intron is removed very much depends on the assortment of trans-acting factors that bind within the vicinity of exon/intron boundaries.[11,12] These splicing regulators modulate the catalytic activity of the spliceosome machinery by interfering with core snRNP: snRNP interactions and/or their recruitment to 5′ splice site (5′SS) and 3′ splice site (3′SS) motifs.[13,14] Consequently, subtle changes in the level/activity of splicing regulators can drive the reaction in either direction at any stage of the splicing process.

Splicing regulators are generally thought to be responsible for coordinating distinct alternative splicing patterns that underpin the functional properties of different cell-types/tissues and influence cell differentiation during development.[15-17] However, evidence has been mounting over recent years highlighting an additional regulatory role for the canonical U-snRNPs in tissue-specific alternative splicing events.[18-21] Significantly, numerous examples of human diseases have been described that are specifically caused by mutations within particular U-snRNA molecules, key U-snRNP biogenesis factors or defects that disrupt pivotal snRNP: snRNP interaction at particular stages of the splicing process. The most surprising characteristic common to all such pathologies is the overwhelming absence of a generalized splicing defect. Instead, for reasons that are not currently well understood, the specific defect impacts distinct cells/tissues.

Disturbing the balance: Mis-regulation of spliceosomal U-snRNP biosynthesis

The biogenesis of U-snRNPs is a highly regulated and intricate stepwise process.[22] Following export of the U-snRNAs into the cytoplasm, a heptapeptide Sm (Smith antigens) ring structure is loaded onto conserved motifs, stabilizing the U-snRNA structure and protecting it from degradation by cytoplasmic nucleases. SnRNA-specific factors are typically assembled onto this structure, following their re-import back into the nucleus, where they exert their function.[23] Considering the ubiquitous nature of these assembly factors and their key involvement in spliceosomal

Table 1. Summary of diseases associated with defects in core spliceosome components.

Disease	Gene	Function	Ref.
SMA (spinal muscular atrophy)	SMN1	SMN complex	24, 25
ALS (amyotrophic lateral sclerosis)	FUS/TLS	RNA binding protein part of the TET family (98)	26, 28-30
CCMS (cerebrocostomandibular syndrome)	SNRPB	Sm protein	33, 34
HS (hypotrichosis simplex)	SNRPE	Sm protein	35
PN (poikiloderma with neutropenia)	USB1	U6/U6atac-specific 3'-5' exonuclease	43, 44, 45
RP (retinitis pigmentosa)	PRPF31	U4/U6 di snRNP	54-58
	PRPF8	U5 snRNP	
	BRR2	U5 snRNP	
	PRPF4	U4/U6 di snRNP	
	PRPF3	U4/U6 di snRNP	
MFDM (mandibulofacial dysostosis microcephaly)	SNU114	U5 snRNP	65
BMKS (Burn-McKeown syndrome)	TXNL4A/DIB1	U5 snRNP	100
Nager syndrome	SF3B4	U2 snRNP	66
		U11/U12 di-snRNP	
MDS/CMML (myelodysplastic syndromes/chronic myelomonocytic leukemia)	SF3B1	U2 snRNP	67, 68
	ZRSR2	U11/U12 di-snRNP	
		U11/U12 di-snRNP	
MODP1/TALS (microcephalic osteodysplastic primordial dwarfism type 1/Taybi-Linder syndrome)	RNU4ATAC	U4atac snRNA	70, 71
Roifman syndrome	RNU4ATAC	U4atac snRNA	72
Cerebellum ataxia	RNU12	U12 snRNA	75
Neuronal degeneration	RNU2	U2 snRNA	76

U-snRNP biogenesis, it is puzzling that de-regulation of the function/location of different components of the snRNP biogenesis pathway lead to distinct human pathologies that exhibit tissue specific defects. At least four different human diseases involved in the snRNP biogenesis pathway, including spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), cerebrocostomandibular syndrome (CCMS) and hypotrichosis simplex (HS) have been described to date (Fig. 1, Table 1).

SMA is possibly the best-known spliceosomal snRNA-associated disease caused by the loss of the survival of motor neuron 1 gene, *SMN1*,[24] which leads to progressive loss of motor function due to deterioration of motor neurons connecting the brain and spinal cord. *SMN1* encodes for the ubiquitously expressed SMN protein, which is part of the SMN multi-protein complex and required for loading the Sm ring structure.[25] ALS is another progressive neurodegenerative disease affecting voluntary muscles in adults. Mutations within several genes are known to cause ALS,[26] including an RNA binding protein known as FUS/TLS (fused in sarcoma/translocated in sarcoma), which is a component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complex and known to participate within multiple steps in RNA metabolism, including transcription, splicing, mRNA transport and translation.[27-30] Although no clear causing mechanism has been described for ALS, mutations in FUS/TLS affect its interaction with the U1 snRNP and abnormally enhance its association with SMN, causing a build up of non-functional aggregates both in the cytoplasm and nucleus.[31,32] The latter two pathologies, CCMS and HS, are the result of mutations within SNRPB (CCMS)[33,34] and SNRPE (HS)[35] Sm proteins, respectively, which have structural roles and assemble onto all spliceosomal U-snRNAs by the SMN complex as outlined above. CCMS and HS are both rare disorders primarily affecting the development of the ribs and facial features including jaw, mouth and tongue[36] and, abnormalities of the hair shaft, respectively. The disparate tissue specificity associated with these diseases is unanticipated considering that all four conditions either disrupt Sm core assembly, which is an essential key step in snRNP biogenesis, or result in the biogenesis of mutant spliceosomal U-snRNPs.[35] Surprisingly, disruption of this assembly machine does not lead to global defects in spliceosomal snRNA/snRNP levels either.[28,35,37-39] Only modest, non-uniform alterations in spliceosomal snRNAs were observed in different cell types. For example, U-snRNAs from the major spliceosome were slightly increased in SMA mice[38] compared to controls. Somewhat reduced levels were quantitated in fibroblasts from ALS patients and spinal cords from FUS transgenic mice[28] and no changes in either U-snRNA or U-snRNP levels were observed in any other cells/tissues analyzed, including lymphocytes from patients suffering from HS.[35] Interestingly, a few reports did observe significant reductions in U11/U12 levels and defects in the formation of the minor U4atac/U5/U6atac tri-snRNP complex.[28,37,40,41] As
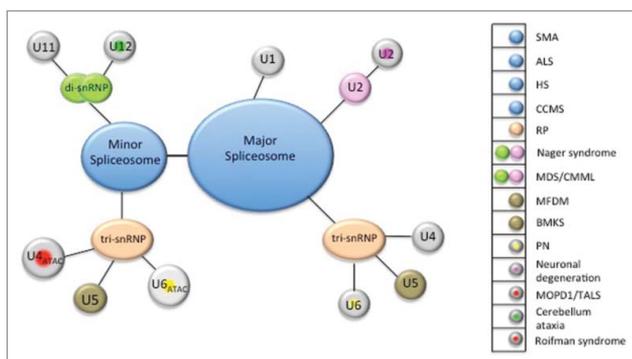
Figure 1. Human pathologies associated with mutations in core spliceosome components. Schematic depicting the core U-snRNP machineries of both the major and minor spliceosome. Diseases are shown on the right with colour discs and with their abbreviated names. Specific defects targeting shared or distinct components of the spliceosome are colour coded (defects that affect all U-snRNPs (Blue), U4/U5/U6 and U4atac/U5/U6atac snRNPs (Orange), U11/U12 di-snRNP (Green), U2 snRNP (Pink), U5 snRNP (Brown), U6 snRNA (Grey/Yellow), U2 snRNA (Grey/Pink), U4atac snRNA (Grey/Red) and U12 snRNA (Grey/Green)). SMA, spinal muscular atrophy; ALS, amyotrophic lateral sclerosis; HS, hypotrichosis simplex; CCMS, cerebrocostomandibular syndrome; RP, retinitis pigmentosa; MDS, myelodysplastic syndromes; CMML, chronic lymphocytic leukemia; MFDM, mandibulofacial dysostosis with microcephaly; BMKS, Burn-McKeown Syndrome[100]; PN, Poikiloderma with Neutropenia; MOPD1, microcephalic osteodysplastic primordial dwarfism type 1.

reported for the major spliceosomal U-snRNAs, the reduction in levels was not uniform across the different cells/tissues analyzed. For example, U11/U12 snRNA levels were significantly reduced in lymphocytes and tissues, including spinal cord, motor cortex, thalamus and liver of SMA/ALS patients, but no changes were demonstrated in cerebellum, skeletal muscle or kidneys.[40,41] In agreement with reduced U11/U12 snRNA levels, splicing of minor introns is impaired in some cells/tissues from affected patients and mouse models.

A separate disorder known as poikiloderma with neutropenia (PN) is caused by defects within a gene responsible for U6 maturation[42] (Fig. 1, Table 1). Unlike the other spliceosomal U-snRNAs, biogenesis of U6 occurs exclusively in the nucleus.[22] The recently discovered USB1 (U6 specific binding 1) 3′-5′ exoribonuclease is responsible for the removal of the terminal uridine residue to form a 2′, 3′, -cyclic phosphate moiety.[43,44] USB1 regulates the stability of U6 by enhancing the assembly of the ring structure, which is composed of Sm-like (LSm) proteins, at the 3′ end of the U6 snRNA. Unlike the yeast orthologue (MPN1), U6atac is also a substrate for USB1 in humans.[45] Myeloid cells are particularly sensitive and defects impact on skin and bone tissues especially.[46,47] While disruption of U6 3' end processing is evident in PN patients and USB1-deficient cells, deep RNA sequencing (RNA-seq) analysis of PN lymphoblastoid cells revealed no significant changes in U6 steady state levels and no obvious defects in splicing.[43,44] In contrast, morpholino inactivation of MPN1 in yeast resulted in global defects in pre-mRNA splicing, which could be reversed upon overexpression of U6.[44] These data demonstrate that the impact of U6 misprocessing varies among different organisms and cell types and the magnitude of the affect could be related to the numbers of U6 gene copies expressed within the different species.

Since the biogenesis/turnover of spliceosomal U-snRNPs is known to vary between different cells/tissues,[48-51] perturbations in the global U-snRNP biogenesis pathway would have a unique impact on relative U-snRNP levels in different cell types. Taking into consideration the rate of transcription and the assortment of splicing regulators in the different cell types, it is thought that subtle changes in U-snRNP compositions could reach critical levels in particular cells/tissues that manifest as a tissue-specific disease pathology.[52]

Mis-regulation of spliceosomal U-snRNP core components

Distinct snRNP: snRNP re-arrangements are required during the splicing process to signal progression through the different stages of intron removal.[53] For example, the first step of intron removal requires binding of the U1 snRNP to the 5' splice site (5' SS) to mark the site of the 1st transesterification event. Following this, a catalytic inactive U6 snRNP is recruited to this region as part of a tri-snRNP complex with the U4 and U5 snRNPs. Activation of the spliceosome complex is achieved following disengagement of the U1 snRNP from the 5' SS, dissociation of the U4 snRNP from the tri-snRNP, enabling the U6 snRNP free to base-pair with the 5' SS and form additional interactions with the U2 snRNP, which is bound at the branch point (BP). After completion of both transesterification events at the 5' SS and 3' SS, respectively, the U2, U5 and U6 snRNPs are released and recycled for additional rounds of splicing. Although the specificities and affinities of the spliceosomal U-snRNPs for distinct snRNP: 5' SS /3' SS /BP and snRNP: snRNP interactions at distinct intronic regions throughout the nascent transcript are often regulated by the cell-/tissue-specific splicing regulators, defects in the expression of any general/specific component(s) of the U-snRNP complexes would still be expected to give rise to general splicing defects. On the contrary, at least five human pathologies have been described to date, including retinitis pigmentosa (RP), myelodysplastic syndromes (MDS), nager syndrome, mandibulofacial dysostosis with microcephaly (MFDM), and Burn-McKeown syndrome (BMKS), which are associated with specific mutations within particular spliceosomal snRNP components with tissue-specific consequences (Fig. 1/Table 1).

Mutations in at least five genes, PRPF31, PRPF8, BRR2, PRPF4 and PRPF3, which are core components of the major and minor tri-snRNP complexes, are associated with RP.[54-58] RP is a disease that causes severe vision impairment due to the progressive degeneration of the rod photoreceptor cells in the retina.[59] The selectivity for this cell type is striking as no degeneration outside the retina has been observed. Where analyzed, none of the mutations affect spliceosomal U-snRNA/U-snRNP levels but significant defects in tri-snRNP assembly, location and U4/U6 unwinding were observed in patient samples and in vitro models established in yeast and human cells.[60-63] It is thought that photoreceptor cells are particularly sensitive, as they are known to express the highest levels of housekeeping genes and have a daily requirement to replenish disc proteins.[64] This high demand in splicing activity maybe specifically regulated by cell-type-specific splicing regulators, which participate during the catalytic stages of the spliceosome, to generate retina-specific transcripts. Consequently, subtle exacerbation in tri-snRNP levels especially, might first manifest in the retina due to imbalances in this particular splicing network.

Having said this, mutations within the gene encoding the U5 snRNP-specific 116K protein (SNU114), which plays an essential role in facilitating remodeling of the tri-snRNP near the catalytic center and would also be predicted to give rise to defects in the retina, is associated with a completely different disorder known as MFDM.[65] MFDM is very similar to CCMS (see above) but associated abnormalities are typically restricted to the head and face only.[36] Interestingly, another acrofacial dysostosis disorder, nager syndrome, which is distinguished from MFDM and CCMS by accompanying upper limb abnormalities, is linked to mutations in a separate protein (SF3B4) that participates at an earlier stage of spliceosome assembly.[66] SF3B4 is one of seven proteins, which form part of a multi-protein complex known as SF3b, and is an integral component of both the U2 and U11/U12 snRNP complexes.[3] SF3B4 is thought to contribute to the stabilization and proofreading of U2 and U11/U12 snRNPs to BP regions within nascent transcripts.

Despite the fact that some proteins share common functions in the splicing process and integral components of the same U-snRNP, different disorders often manifest when their activity is dysregulated. For example, the gene encoding SF3B1, another member of the SF3b complex, has been described as among the most highly mutated in a group of neoplasms known as MDS.[67] These diseases are characterized by dysplasia in blood cell production predisposing to acute myeloid leukemia (AML) or chronic myelomonocytic leukemia (CMML). Recurrent, mutually exclusive

mutations in ZRSR2, which is a component of the U11/U12 di-snRNP, are also frequently associated with this group of neoplasms.[68,69] The reasons why defects in these key spliceosomal U-snRNP components would lead to such narrow tissue-specific consequences are currently not clear. It is likely that some splicing regulators maybe particularly sensitive to variations in the activity of the corresponding U-snRNP partner. This would lead to switching between alternative splice sites that would manifest in a pathology with tissue-specific abnormalities, if the expression of the splicing regulatory in question was restricted to a particular cell-type or stage of development, for example. Moreover, the mutant U-snRNP may have a distinct function of its own that may influence a specific disease phenotype.

Mis-regulation of canonical U-snRNAs

In addition to mutations that alter protein components of the core spliceosome, mutations arising within the core spliceosomal snRNAs also underlie a discrete set of diseases, including microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1), Roifman syndrome and ataxia, including general neurodegenerative ataxia and early onset cerebellum ataxia. Considering that spliceosomal U-snRNAs are at the heart of the spliceosome machinery and play pivotal roles in re-shaping the transcriptome in all of our cells, it is astonishing that abrogation in function is compatible with growth and development of most tissues. Mutations within U4atac snRNA are associated with two similar but distinct rare development disorders, including MODP1, also known as Taybi-Linder syndrome (TALS), and Roifman syndrome.[70-72] Both diseases contain mutations within a stem loop region that specifically interferes with binding of a core component of the U4atac/U6atac (as well as U4/U6) snRNP duplexes, known as 15.5K. Additional mutation(s) within the conserved Sm site or the region involved in base pairing with U6atac is associated with Roifman syndrome. In addition to growth retardation, neurological defects, and malformations of the face and joints, severe retinal dysplasia and immunodeficiency distinguish Roifman syndrome from MOPD1. Surprisingly, none of the mutations described alters U4atac snRNA levels in patient cells, including umbilical cord fibroblast or MOPD1-derived pluripotent cells.[73] However, significant reductions in minor spliceosome assembly were evident in assays targeting different proteins of the tri-snRNP complex. For example, no change in the assembled U4atac snRNA levels were observed in Sm immunoprecipitations, whereas drastic variations in levels were quantitated using antibodies targeting different proteins, including 110K, 100K and PRPF31. The fact that subtle changes in the composition of the U4atac snRNP, arising from variation in the affinities for U-specific factors with the different mutants, can manifest into distinct pathologies supports the notion that individual spliceosomal U-snRNAs have, as yet uncharacterized, additional roles in splicing regulation.

The observation that defects associated with the minor spliceosome are commonly linked to diseases of the central nervous system, including SMA, suggest that this machinery may have a distinct regulatory role in controlling neurogenesis.[74] In support of a link between defects of the minor spliceosome and diseases of the central nervous system, a study in mouse demonstrated that minor U-snRNAs are particularly enriched in the developing nervous system and inactivation of their activity, using U-snRNA-specific morpholinos, resulted in terminal defects in neuronal differentiation and survival.[50] Moreover, another more recent study in humans demonstrated that a singe point mutation within the minor spliceosomal U12 gene (RNU12) was the underlying cause of early onset cerebellum ataxia.[75] The mutant U12 snRNA is thought to specifically interfere with the binding of the U11/U12-specific 65K protein and disrupt assembly of the U11/U12-di-snRNP. RNA-seq analysis of patient blood mononuclear cells indicated a significant enrichment in minor intron retention particularly in RNA substrates known to be associated with pathways relevant to neurological disease, embryonic development, and organ morphology.

Interestingly, total U12 snRNA levels were elevated in ataxia patient blood mononuclear cells compared to healthy controls. This change in U12 snRNA levels suggests there exists a quality control mechanism regulating the correct balance of normal spliceosomal U-snRNA levels and disruption of snRNA levels, in different cell types, could lead to a tissue specific pathology. In support of disrupted U-snRNAs levels associated with tissue specific pathologies, a study in mice showed that a 5 bp deletion within one member of a cluster of U2 snRNA genes causes progressive neurodegeneration of the brain with neuron death most severe in the cerebellum.[76] The deletion removes the first two nucleotides of the U2 BP recognition sequence (BSRS) and 3 nucleotides within the region linking the BPRS and U2/U6 helix and would be predicted to have a global defect in SS recognition. Interestingly though, the expression of this particular mutant U2 gene is temporally and spatially regulated, reaching ~50% of total U2 snRNA levels in the cerebellum and levels coincide with the onset of neurodegeneration. Exon array and RNA-seq analysis of wildtype and mutant mouse cerebella indicated an increased retention of small introns, in particular, containing weak splice sites in a manner coincident with expression of the mutant U2 snRNA. Consequently, the outcome of the disease is thought to be the consequence of normal U2 levels reaching critical levels in the cerebellum and the mutant U2 unable to fully function on the suboptimal splice sites located within the RNA substrates generated in this particular tissue. In addition, this discrete regulation of a mutant U2 gene, which is a member of a large U2 multi-gene family,[77,78] suggests that post-transcription mechanisms that are responsible for U-snRNP biosynthesis, in particular, play important role(s) in regulating U-snRNP function in different cell types.

Compensatory mechanism resets the balance

One aspect of the spliceosome machinery that has been particularly overlooked in recent years is the existence of abundant, evolutionary conserved, non-canonical U-snRNA gene copies. The majority are thought to have a limited and even negligible biological function and have been awarded the label of non-functional pseudogenes. However, in recent years this so-called non-functional genomic dark matter has been shown to encode an immense assortment of distinct regulatory RNA molecules, which are essential in governing key stages of cell differentiation and development.[79,80] Significantly, these non-coding
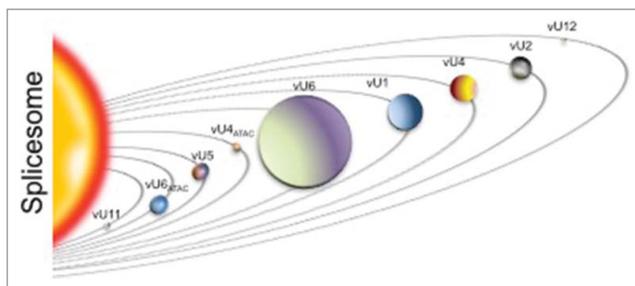
Figure 2. Potential new players in the spliceosome system. Current annotations of the human genome indicate that up to 1,300 variant gene copies exist for each spliceosomal U-snRNA. In the above schematic, different spliceosomal snRNA genes are each denoted by a sphere. The size of each sphere is representative of the number of variant(v) gene copies (141 vU1, 71 vU2, 90 vU4, 37 vU5, 1,300 vU6, 4 vU11, 1 vU12, 18 vU4atac and 42 vU6atac gene copies). The existence of these vU-snRNA genes copies, their regulation and mis-regulation in development and disease, respectively, and their potential involvement in the spliceosome machinery, suggests an additional layer of regulation that has gone unnoticed for decades. We propose that changes within the stoichiometry and composition of U-snRNP repertoires underlie the tissue specific phenotypes associated with different pathologies arising from defects in core spliceosomal components.

RNAs often function in processes that directly or indirectly control the expression of other molecules thus offering an additional level of regulation and a potential therapeutic target.

The human genome encodes up to 1,300 variant gene copies of each spliceosomal U-snRNA (Fig. 2). Many contain base changes and/or small deletions/insertions throughout the body of the U-snRNA encoding region, including sites important for base pairing interactions with the pre-mRNA and/or within binding motifs recognised by specific factors.[81-84] Importantly, evidence accumulating over recent years demonstrate that these gene products are functional, as non-canonical spliceosomal U-snRNAs mapping to these variant gene sequences are assembled into RNPs and, in the case of U5 and U1 snRNAs, incorporated into spliceosomal particles that participate in mRNA processing events, respectively.[81-83] As expected, many vary in their affinities for specific factors and some form high molecular weight assemblies that differ substantially from their corresponding canonical U-snRNA. For example, we and others have shown that variant (v)U1 snRNAs, in particular, associate with core U1 snRNP proteins to a lesser extent than the canonical U1 and some variants at least can form novel snRNP complexes lacking U1-70k and U1-A proteins altogether.[83,85] Interestingly, few base changes are observed at the Sm binding motif. Altering the binding sites, while maintaining the Sm motif, would ensure the proper biogenesis of multiple vU1 snRNP complexes with different specificities/affinities for components of the spliceosome machinery.[86]

Unlike the canonical U-snRNPs, which are constitutively expressed, all expressed variants studied to date appear to be differentially regulated.[87] Several studies in different organisms have revealed that different forms of U-snRNAs are expressed in the unfertilized and fertilized eggs, during development and in fetal and adult tissues.[88-92] Some U1 variants, for example, can account for up to 40% of total U1 snRNA levels during the early stages of development.[90] Furthermore, expression of the non-canonical U-snRNAs typically follows a distinctive inversed pattern compared to their corresponding canonical U-snRNA. For example, variant U1 snRNAs are highly expressed in human pluripotent stem cell (PSC) lines and

significantly down regulated in differentiated cell-types including monocytes and neurons.[48] In contrast, U1 snRNA levels are significantly reduced in PSCs and smooth muscle progenitors.[48,49] Similarly, changes in U5/vU5 snRNA ratios and temporal expression patterns were also observed during fly and sea urchin development.[81,93] Importantly, the differential expression appears to have implications in the maintenance of pluripotency as NANOG protein levels are significantly increased following ectopic expression of vU1 snRNAs in human skin fibroblasts. Notably, this increase in NANOG levels was only detected when a combination of U1 variants at a specific concentration was used. These data strongly support the notion that vU-snRNAs have important physiological function(s) in cells and that regulatory systems are in place to ensure that precise canonical: non-canonical U-snRNA ratios are monitored and maintained in appropriate cell types. In support of this notion, we recently showed that disruption of the U1/vU1 snRNA ratio is associated with the neuropathology of SMA disease.[48] U1/vU1 snRNA ratios are notably altered in PSC-derived motor neuron cultures (MNs) from patients compared to healthy control subjects, in favour of the vU1 snRNAs. This change in ratio was specific to SMA disease and MN cultures in particular, as no change in ratios was observed in healthy controls and Parkinson patient fibroblasts and patient-derived dopaminogeneric neurons.

RNA surveillance pathways involving terminal uridyltransferases (TUT1/4/7 and GLD−2)[94] and 3′-5′ exonucleases (DIS3L2 and DIS3) have recently been reported to form part of a general mechanism of snRNA quality control and degradation in mammalian cells.[95,96] However, the mechanism(s) maintaining balance across spliceosomal U-snRNPs repertoires in different cell types are currently not known. Evidence exists highlighting the importance of variant-specific promoter elements in driving differential expression of U6 snRNA variants and developmental regulation of mouse U1 genes, for example.[84,97,98] However, post-transcription events including competition for general biogenesis factors and splicing regulators are likely the key modulators of spliceosomal U-snRNP repertoires and their relative abundance in different cell types. For example, expression of the RNU2-8 mutant, which is encoded by an individual member of the U2 multi-gene family, is unequally distributed in different mouse tissues with levels comprising 45% of total U2 snRNA levels in the cerebellum and significantly reduced in all other tissues analysed.[76] It's possible the RNU2-8 mutant forms a more stable complex in the cerebellum due to novel associations of a tissue specific splicing regulator with the mutated region.

It would seem that the trade-off in maintaining appropriate relative levels of spliceosomal U-snRNPs repertoires in different cells could come at a cost for some cell-types, in particular. Although changes in U-snRNA repertoires could explain the tissue-specific phenotype of pathologies described above that involve defects in specific U-snRNA molecules, including MODP1 and cerebellum ataxia for example, it does raise questions as to how overall levels of U-snRNA/RNPs are only minimally affected following de-regulation of essential factors required for biosynthesis and activity. Considering that many non-canonical U-snRNAs are differentially expressed and generate snRNA products with high sequence similarities to

canonical genes, it's possible that the way we measure U-snRNA levels has meant that conclusions drawn from the previous studies may have been erroneously assigned to the particular U-snRNA under investigation.[99] For example, several techniques including knockdown studies and quantitative PCR analysis, to mention a few, rely heavily on the specificity of gene specific primers and often assume that corresponding variant U-snRNA gene copies are inactive and thus would not contribute to the particular analysis used. In light of what we now know regarding the mis-regulation of vU-snRNAs in disease models, their existence could explain why U-snRNA levels remain relatively unchanged in the majority of disease models outlined above. In support of vU-snRNAs acting as counterbalance of U-snRNA levels in disease models, drastic reductions in assembled U-snRNAs are only observed in radiolabelled/biotinylated assembly assays that specifically target individual U-snRNAs for analysis.[37,38] Since many variant U-snRNAs have the potential to recognize non-canonical splice junctions and/ or regulatory motifs, and vary in their affinities for specific RNP factors and/or general/tissue-specific splicing regulators, any imbalances in canonical/non-canonical U-snRNP repertoires would cause the U-snRNP repertoire to change in a distinct way. The resulting perturbations would have an impact on cell-type specific splicing events that would uniquely contribute to the disease phenotype.[83] In agreement with this idea, RNA-seq data from spinal cords extracted from the SMA mouse model indicate a high proportion of aberrant splicing defects, including RNA isoforms containing non-canonical splice site junctions and novel RNA isoforms that do not conform to normal splicing algorithms.[37] These data suggests that the outcome of any disease associated with defective spliceosome core components could be due to compensatory mechanisms such as enhanced expression of the complex array of variant U-snRNA genes currently annotated in our genome (Fig. 2). This change in the snRNP repertoires, which has been overlooked until now, could be a major contributing factor to the tissue-specific outcomes characteristic of diseases associated with loss of core splicing factors.

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