Published in final edited form as:
Semin Cell Dev Biol. 2014 August ; 0: 22–29. doi:10.1016/j.semcdb.2014.04.026.

SMN control of RNP assembly: from post-transcriptional gene regulation to motor neuron disease

Darrick K. Li[1,2], **Sarah Tisdale**[1,2], **Francesco Lotti**[1,2], and **Livio Pellizzoni**[1,2,*]

[1]Center for Motor Neuron Biology and Disease, Columbia University, New York (NY) 10032, USA

[2]Department of Pathology and Cell Biology, Columbia University, New York (NY) 10032, USA

Abstract

At the post-transcriptional level, expression of protein-coding genes is controlled by a series of RNA regulatory events including nuclear processing of primary transcripts, transport of mature mRNAs to specific cellular compartments, translation and ultimately, turnover. These processes are orchestrated through the dynamic association of mRNAs with RNA binding proteins and ribonucleoprotein (RNP) complexes. Accurate formation of RNPs *in vivo* is fundamentally important to cellular development and function, and its impairment often leads to human disease. The survival motor neuron (SMN) protein is key to this biological paradigm: SMN is essential for the biogenesis of various RNPs that function in mRNA processing, and genetic mutations leading to SMN deficiency cause the neurodegenerative disease spinal muscular atrophy. Here we review the expanding role of SMN in the regulation of gene expression through its multiple functions in RNP assembly. We discuss advances in our understanding of SMN activity as a chaperone of RNPs and how disruption of SMN-dependent RNA pathways can cause motor neuron disease.

Keywords

Spinal Muscular Atrophy (SMA); Survival Motor Neuron (SMN); small nuclear RNA (snRNA); ribonucleoprotein complexes (RNPs); Sm and LSm proteins; RNA processing

1. Introduction

The eukaryotic genome is transcribed into RNA comprising protein-coding mRNAs and non-coding RNAs (ncRNAs) that are critical for regulation of gene expression. A hallmark of both mRNAs and ncRNAs is their association with specific RNA binding proteins (RBPs) to form ribonucleoprotein complexes (RNPs) in the context of which they perform their diverse roles in gene expression. Formation of RNPs often involves multi-step pathways with precise spatial and temporal coordination. Reflecting the key role of RNP biology, a

*To whom correspondence should be addressed: Center for Motor Neuron Biology and Disease, Department of Pathology and Cell Biology, Columbia University, Physicians & Surgeons Building, Room 5-421, 630 West 168th Street, New York, NY 10032, USA, Phone:+1-212-305-3046, Fax: +1-212-342-0276, lp2284@cumc.columbia.edu.

dramatic expansion of the ncRNA transcriptome has accompanied the evolution of organism complexity [1], and a growing list of human diseases—a large proportion of which affect the nervous system—are caused by mutations in ubiquitously expressed genes implicated in RNA processing and post-transcriptional gene regulation [2].

The myriad of RNAs and the large number of RBPs in eukaryotic cells raises the fundamental biological question of how proper formation of RNPs is orchestrated in the complex cellular microenvironment. Studies of the biogenesis of essential RNP components of the splicing machinery revealed that RNP assembly is an assisted process in which the survival motor neuron (SMN) protein functions as a molecular chaperone [3]. Ubiquitous SMN deficiency causes spinal muscular atrophy (SMA)—an inherited disorder characterized by the degeneration of spinal motor neurons and atrophy of skeletal muscle [4]. SMN has therefore become a model for understanding the role of chaperone-mediated RNP assembly in RNA processing and the contribution of RNA dysfunction to neurodegeneration.

This review focuses on the activity of SMN as a molecular machine for the formation of diverse cellular RNPs, its critical role in post-transcriptional gene regulation, and the consequences of its loss for the development and function of the motor system. Recent studies have significantly advanced our knowledge of the molecular mechanisms by which SMN functions as an RNP assembly chaperone and how its deficiency causes SMA. Beyond unraveling basic aspects of RNA regulation and SMA pathology, the study of SMN biology has addressed the broader question of how defects in ubiquitously expressed RNA processing factors cause selective dysfunction of specific subsets of neurons—a fundamental problem common to many degenerative disorders of the nervous system.

2. The SMN complex

The *SMN* gene was identified in the mid-1990s, the culmination of an extensive search for the gene responsible for SMA [5]. The SMN protein is an evolutionarily conserved and ubiquitously expressed protein that localizes to both the cytoplasm and the nucleus where it accumulates in nuclear structures known as Gems. The discovery that Gems are associated with Cajal bodies (CBs)—nuclear domains implicated in the assembly and modification of RNPs—provided the first hint of SMN's involvement in RNA regulation [6]. SMN associates with eight other proteins (Gemins2-8 and Unrip) to form a large macromolecular complex through a network of reciprocal interactions (Figure 1) [3 and references therein]. A key feature of the SMN complex is its ability to form higher-order particles ranging in size from 20S to 80S [7, 8]. Rather than differential association of its integral components, the heterodispersed nature of these complexes likely reflects the self-oligomerization properties of SMN. SMN oligomerization requires the carboxy-terminal, evolutionarily-conserved YG-box and is disrupted by SMA-associated missense mutations in SMN [9, 10], indicating self-association as a key element for SMN function. Recent studies revealed the structural basis of SMN oligomerization by showing that the YG-box can form helical oligomers mediated by glycine zippers similar to those found in transmembrane channel proteins [11]. SMN contains additional evolutionarily conserved regions involved in protein-protein interactions, including a Tudor domain that binds symmetrically dimethylated

arginines found within many SMN binding proteins [12]. These protein interaction domains likely contribute to forming the core scaffold upon which the different components of the SMN complex are assembled. Future determination of the stoichiometry of the individual subunits of the SMN complex and further structural information about their interactions will continue to reveal the inner workings of this dynamic multiprotein machine.

How the SMN complex is assembled is unknown, but several of its integral components are also found in distinct multi-protein complexes comprised of Gemin3/4, Gemin6/7/Unrip, and Gemin5, either alone or in association with Gemin3/4 [7, 8]. These observations suggest that the SMN complex may undergo stepwise assembly through a series of modular additions. They may also reflect a steady growth in complexity of the complex throughout evolution. Accordingly, an ancestral version of the SMN complex in fission yeast comprised of only SMN and Gemin2 evolved through the stepwise addition of Gemin proteins to the multisubunit, human SMN complex [13]. This recruitment of new components to the SMN complex may reflect an evolutionary increase in the complexity of the pathway in which it functions, allowing more precise regulation and the acquisition of additional properties that underlie secondary functions. Nevertheless, SMN and all Gemins tested to date are essential for viability in divergent organisms from yeast to mouse [4], indicating that they perform essential cellular functions.

3. The SMN complex functions in snRNP assembly

Studies of SMN function revealed an unexpected role for SMN in the biogenesis of small nuclear ribonucleoproteins (snRNPs) involved in distinct RNA processing pathways. Through functions in RNP assembly, the SMN complex is required for the expression of essentially all protein-coding genes.

3.1. The biogenesis pathway of spliceosomal snRNPs

Spliceosomal snRNPs remove introns from pre-mRNA through two trans-esterification reactions mediated by a large machine known as the spliceosome. The majority of introns are processed by the major (U2-dependent) spliceosome comprised of U1, U2, U4/U6, and U5 snRNPs while a small group of introns are processed by the minor (U12-dependent) spliceosome comprised of U11, U12, U4atac/U6atac, and U5 snRNPs. With the exception of U6 and U6atac, each spliceosomal snRNP consists of a U-rich small nuclear RNA (snRNA), a set of seven Sm proteins (B/B′, D1, D2, D3, E, F, and G) and additional snRNP-specific proteins [3, 14]. The biogenesis of snRNPs is a complex process that involves both nuclear and cytoplasmic phases.

Biogenesis begins in the nucleus with transcription by RNA polymerase II (pol II) of precursor snRNAs (pre-snRNAs) that are co-transcriptionally modified at their 5′-end to include a 7-methyl guanosine (m^7G) cap and cleaved at their 3′-end by the Integrator complex [14], which yields a pre-snRNA with an extra stem-loop structure at the 3′-end [15]. Following transcription, a multi-protein export complex assembles onto pre-snRNAs [3, 14]. First, the heterodimeric cap-binding complex (CBC) comprised of CBP20 and CBP80 associates with the m^7G cap. Second, the phosphorylated adaptor for RNA export (PHAX) is recruited through interactions with CBC and the snRNA. PHAX is then

recognized by the export receptor Exportin 1 (Xpo1) bound to RanGTP to form a functional nuclear export complex. Recent studies expanded the repertoire of cellular proteins involved in snRNA export complex formation to include ARS2, which stimulates PHAX binding to CBC and snRNA 3′-end processing [16], and a heterodimer of the RNA-binding proteins p54nrb/NonO and PSF that promotes loading of snRNA-specific export factors [17].

Upon cytoplasmic entry, PHAX dephosphorylation and GTP hydrolysis trigger dissociation of Ran and Xpo1 from the pre-snRNA followed by association of the SMN complex with pre-snRNAs still bound to CBC and dephosphorylated PHAX [18]. The SMN complex then mediates the ATP-dependent assembly of a heptameric ring of Sm proteins (Sm core) around a conserved uridine-rich sequence of each snRNA (Sm site) [19, 20]. To do this, the SMN complex interacts with both Sm proteins and snRNAs through a highly ordered series of events requiring association with the seven Sm proteins before snRNA binding (Figure 1) [19–22], which is mediated by Gemin5 [23]. Prior to their binding to the SMN complex, Sm proteins associate with both the chloride conductance regulatory protein (pICln) and the protein arginine methyltransferase 5 (PRMT5) complex [24, 25] where a subset of Sm proteins (B/B′, D1, D3) undergo symmetrical dimethylation of arginine residues that increases their binding affinity for SMN *in vitro* [26, 27]. The SMN complex preloaded with the Sm proteins represents the molecular entity poised to associate with newly exported pre-snRNAs for Sm core assembly [19, 20]. This key event is required for the subsequent steps of snRNP biogenesis, including m^7G-cap hypermethylation by TGS1 and snRNA 3′-end trimming. The newly assembled SMN-bound snRNP is then imported back into the nucleus through the binding of Snurportin-1 to the trimethylated 5′ cap and the association of both Snurportin-1 and SMN with importin β [18]. Interestingly, recent work has highlighted a cytoplasmic quality control mechanism for snRNP biogenesis, where SMN-associated snRNAs that cannot be assembled with an Sm core are diverted to cytoplasmic P bodies for degradation [28]. Upon entry in the nucleus, the import complex dissociates and the SMN-bound snRNPs transiently localize to CBs—where snRNAs undergo 2′-*O*-methylation and pseudouridylation by CB-specific scaRNPs and associate with individual snRNP-specific proteins—before mature snRNPs join the spliceosome to function in pre-mRNA splicing.

3.2. The biogenesis pathway of U7 snRNP

In addition to spliceosomal snRNPs the SMN complex is required for the assembly of U7 snRNP, which functions in the 3′-end processing of histone mRNAs. Metazoan replication-dependent histone genes are intronless and non-polyadenylated. Histone transcripts terminate in a highly conserved 3′-end stem-loop structure essential for their proper cell cycle regulation [29]. The unique 3′-end of histone mRNAs is generated by a single endonucleolytic cleavage that requires base pairing of U7 snRNA with a conserved sequence located in the 3′ UTR of histone mRNAs downstream of the cleavage site. Together with the stem-loop binding protein (SLBP) that binds to the upstream 3′ hairpin structure, U7 snRNP promotes the recruitment and proper positioning of *trans*-acting factors for cleavage of histone transcripts [29].

U7 snRNP follows a biogenesis pathway analogous to that of spliceosomal snRNPs, with several important differences. U7 snRNA contains a slightly degenerate Sm site compared to

the canonical Sm site of spliceosomal snRNAs [30], and the unique heptameric Sm core assembled onto this site contains the Sm-like (LSm) proteins LSm10 and LSm11 instead of SmD1 and SmD2 [31, 32]. The presence of U7-specific LSm proteins is essential for U7 snRNP function as they facilitate important interactions that stabilize U7 onto histone mRNA targets and recruit factors that catalyze cleavage [30]. Remarkably, despite functional and compositional differences from spliceosomal snRNPs (Figure 1), the SMN complex also mediates LSm/Sm core assembly onto U7 snRNA [31, 33]. A distinct SMN complex specialized in the ATP-dependent assembly of U7 snRNP has been identified containing both LSm10 and LSm11 in addition to SmB/D3/E/F/G proteins [31]. Akin to spliceosomal snRNAs [15], the assembly reaction occurs on U7 pre-snRNAs with an additional 3′-end stem-loop structure [33]. Consistent with a requirement of SMN in U7 snRNP biogenesis, SMN deficiency causes the accumulation of U7 pre-snRNA, reduction in U7 snRNP levels and disruption of histone mRNA 3′-end processing [33].

While it is established that distinct SMN complexes exist that are associated with Sm or LSm/Sm proteins for the assembly of spliceosomal snRNPs and U7 snRNP, respectively, how these separate complexes selectively associate with their corresponding snRNAs is unclear. Inaccurate association would likely have detrimental functional consequences. In support of this, and highlighting the critical issue of specificity in snRNP assembly, changing the unique Sm site of U7 snRNA to the Sm site consensus sequence of spliceosomal snRNAs results in the formation of U7 snRNPs with the canonical spliceosomal Sm core, which accumulate abundantly in the nucleus but are non-functional in histone mRNA processing [30, 31]. Although the SMN complex identifies spliceosomal snRNAs through Gemin5 [23], it has not been reported to interact directly with U7 snRNA. Understanding the mechanism that underlies the selectivity of distinct SMN complexes for their cognate RNAs will provide fundamental insights into the principles guiding the fidelity of RNP formation.

4. SMN and the specificity of snRNP assembly

The cellular milieu presents a crowded meshwork of proteins, nucleic acids, cytoskeletal filaments and organelles that decreases the effective volume through which constituents of an RNP freely diffuse. This crowding effectively increases the concentration of macromolecules and promotes both specific and non-specific interactions [34]. Therefore, the complex microenvironment of the cell likely makes the efficient and specific assembly of the RNA and protein constituents of RNPs especially difficult. Early experiments showing that complex RNP machines including the ribosome and spliceosomal snRNPs could be reconstituted *in vitro* from their purified RNA and protein constituents indicated that self-assembly was a major path in the formation of these macromolecular complexes. Studies of SMN complex function in snRNP biogenesis challenged this view and provided direct evidence that RNP assembly is an assisted process requiring protein chaperones *in vivo*.

Purified Sm proteins had been shown to spontaneously associate *in vitro* with snRNAs to form snRNPs structurally analogous to their *in vivo* counterparts [35]. The discovery that the seemingly spontaneous process of snRNP assembly required the SMN complex was

therefore a puzzle up until the realization that Sm proteins have an intrinsic lack of specificity with respect to RNA substrates. The propensity of Sm proteins to form Sm cores readily on any short, single-stranded and uridine-rich sequence [35], led to the finding that the SMN complex is required to prevent illicit binding of Sm proteins on other RNAs and to direct the accurate formation of Sm cores on snRNAs [20]. Thus, the SMN complex functions as a molecular chaperone of RNPs by imposing efficiency and specificity on the process of snRNP biogenesis *in vivo*. This key proofreading activity is evolutionarily conserved in SMN complexes of simpler composition from divergent organisms [13, 36]. The fundamental conceptual advance emerging from these studies established SMN as a paradigm molecular chaperone of RNPs.

Molecular and structural studies have provided fundamental insights into the mechanisms of chaperone-mediated snRNP assembly. On one hand, the SMN complex imposes specificity on snRNP assembly through Gemin5 binding to specific regions of the snRNAs [15, 20–23]. On the other hand, the SMN complex recruits the seven Sm proteins through critical interactions with pICln and the PRMT5 complex. To avoid non-specific associations that might have deleterious consequences on the biology of various RNAs, newly translated Sm proteins are thought to associate first with pICln and the PRMT5 complex and then be transferred to the SMN complex so that no free pool of Sm proteins is found outside of these complexes [24, 25]. A 6S ring-shaped intermediate of snRNP assembly containing pICln associated with the hetero-oligomers SmD1/D2, and SmE/F/G has been characterized structurally [37]. Within this 6S complex, pICln acts as a structural mimic of the SmB/D3 oligomer [38]. Importantly, pICln occupies an angular space that spans the width of 1.5 Sm proteins, resulting in a torus with a significantly narrower and sterically occluded central pore that prevents Sm proteins from accessing snRNAs [38]. To permit snRNP assembly, the Sm pentamer is transferred to the SMN complex with concomitant displacement of pICln [37]. Transient docking of the SMN complex with the outer surface of the 6S pICln-containing complex promotes the destabilization of pICln's interactions with the Sm proteins [38, 39]. At some stage during this transfer, Gemin2 forms extensive interactions with all five Sm proteins, forming strong attachments from both the exterior and the interior of the Sm pentamer [39]. Importantly, Gemin2 maintains the narrow angles of the Sm pentamer and also reaches the RNA-binding domain to prevent spurious association with RNA [39]. Thus, these molecular and structural studies of snRNP assembly reveal elegant strategies employed by RNP chaperones to ensure specificity in the assembly of the snRNP particle. The SmB/D3 heterodimer is likely recruited from the PRMT5 complex independently of the Sm pentamer [24] and thus joins the SMN complex through a different path. It remains unclear how Sm core formation occurs after both Sm protein recruitment and snRNA binding by Gemin5. This likely requires major structural rearrangements, including the release of Gemin2 constraints on RNA binding of Sm proteins, their association with the Sm site, and ring closure by addition of SmB/D3.

5. Expanding roles of SMN in RNA regulation

Numerous lines of evidence place SMN centrally in the biogenesis of diverse RNPs and consequently a wide variety of RNA processing pathways through interactions with various RBPs (Figure 1).

The Sm and LSm proteins form an evolutionarily conserved family of ubiquitously expressed RBPs found in all three branches of life [40], which function in diverse RNA pathways through their ability to form heteromeric, ring-shaped complexes of varying composition that associate with distinct RNAs. Through its activity in Sm core assembly on spliceosomal snRNAs [19, 20], the SMN complex is essential for the synthesis of the key constituents of both U2 and U12 splicing machineries and therefore the expression of intron-containing eukaryotic genes (Figure 1). The recent discovery of novel functions of the U1 snRNP in the suppression of cryptic polyadenylation sites and in the regulation of promoter directionality [41, 42] positions SMN as a potential key regulator of these events as well. Importantly, studies on variations of SMN's canonical function in Sm core assembly have established a more general role for SMN in RNP assembly. For instance, the assembly of Sm cores on viral noncoding RNAs encoded by *Herpesvirus saimiri* (HSURs) is SMN dependent [43]. Additionally, SMN is required for the assembly of the hybrid Sm/LSm core of U7 snRNP essential for 3′-end processing of metazoan histone mRNAs (Figure 1) [31, 33]. These studies provide compelling support for the concept of the SMN complex as the general assembly machine for RNPs of the Sm/LSm protein family.

Two functionally and structurally distinct rings containing only LSm proteins have been identified to date [40]: a nuclear LSm2-8 complex and a cytoplasmic LSm1-7 complex (Figure 1). The LSm2-8 complex binds the uridine-rich 3′-end of U6 and U6atac snRNPs to facilitate U4/U6 di-snRNP formation and snRNP recycling. The cytoplasmic LSm1-7 complex functions in the regulation of mRNA turnover through association with the 3′-end of target transcripts and the recruitment of decapping factors for their 5′ to 3′ degradation. Several observations support SMN's involvement in the biology of these LSm complexes: the similar basic structural organization of Sm and LSm rings, the established role of SMN in Sm and Sm/LSm core assembly, and SMN binding to both LSm4 and LSm6 [26, 44]. Future studies will determine whether the SMN complex is the ringmaster of all Sm and LSm-containing complexes.

In further support of an expanded role in RNA regulation, SMN has been shown to interact with many other RBPs [45]. A large number of these are mRNA-binding proteins implicated in multiple aspects of post-transcriptional gene regulation [46–50], including mRNA transport, stability and local translation in neurons. These RBPs bind SMN directly through their RG-rich domains in a methylation-dependent manner that is often disrupted by SMA-linked mutations of SMN [46–50]. In cultured neurons, SMN localizes with mRNA-binding proteins like hnRNP R, HuD, KSRP and IMP1 in axonal and dendritic granules that exhibit rapid, bidirectional movement [46–48, 50–52]. These granules also contain other integral components of the SMN complex but not Sm proteins [52], suggesting a function for SMN unrelated to snRNP assembly. Importantly, SMN deficiency decreases localization of these RBPs and several associated transcripts in axons and growth cones of developing neurons [46, 48, 51]. Consistent with the requirement for local translation of mRNAs in neuronal pathfinding, these SMN-deficient neurons display reduced neurite length and smaller growth cones [46, 48, 51]. Altogether, these findings suggest that SMN may contribute to neuronal mRNA trafficking perhaps by facilitating the interaction of RBPs with their mRNA targets (Figure 1).

Several SMN-associated RBPs are also involved in other aspects of mRNA regulation and associate with hundreds of transcripts, many of which contain AU-rich elements (AREs) in their 3′-UTRs that are key for the regulation of their turnover. One well-described example is Cdkn1a mRNA, which is subject to antagonistic regulation by both KSRP and HuD and accumulates upon SMN deficiency due to increased stability [49, 50]—a particularly prominent event in SMA motor neurons [53]. Thus, SMN may play a role in the cytoplasmic turnover of transcripts such as ARE-containing mRNAs by modulating their association with RBPs (Figure 1). However, the molecular function(s) of SMN in the biology of mRNPs and other RNPs not described here is unknown and awaits the development of specific assays to provide solid mechanistic insights into the full spectrum of SMN-mediated RNA regulation.

6. SMN-dependent RNA pathways and motor neuron disease

As SMN functions in many essential RNA pathways, abrogation of any of these would be expected to have deleterious effects. Accordingly, SMN knockout is invariably lethal in all cellular and animal models tested to date. Importantly, ubiquitous reduction but not ablation of SMN function in humans results in SMA—a motor neuron disease caused by homozygous inactivation of the *SMN1* gene with retention of two or more copies of the hypomorphic *SMN2* gene [5]. *SMN2* cannot compensate for the loss of *SMN1* because it produces low levels of SMN due to skipping of exon 7—the inclusion of which is critical for the stability and function of the SMN protein [4]. SMA displays a spectrum of clinical severity, the most common and severe form characterized by motor neuron degeneration and progressive skeletal muscle atrophy shortly after birth resulting in paralysis and ultimately death.

SMA has been modeled in many organisms, which has been instrumental to our understanding of the key requirement for SMN-dependent RNA regulation *in vivo* and of the molecular basis of the disease. To date, mouse models of SMA with two copies of *SMN2* best recapitulate the human condition genetically and phenotypically [4]. These mice exhibit motor neuron death and neuromuscular junction (NMJ) defects, including progressive skeletal muscle denervation, consistent with features of human pathology. Remarkably, recent studies have highlighted functional disruption of the sensory-motor circuit as an early pathological event in animal models of SMA [54, 55], pointing to dysfunction of neuronal networks as a key element in the disease. Thus, increasing evidence indicates that SMA pathology is not restricted to motor neurons but rather is the composite of pathology in many cell types.

A fundamental question in SMA biology is how selective motor system dysfunction results from reduced levels of the ubiquitously expressed SMN protein. It is generally agreed that SMA is a loss-of-function disease resulting from dysregulation of RNA metabolism induced by SMN deficiency in disease-relevant cells. However, there is considerably less consensus on which RNA pathways are most salient to disease pathogenesis. SMA likely represents the synergistic convergence of multiple deficits in RNA regulation induced by SMN deficiency. Here we discuss the necessary requirements to establish the relevance of any SMN-

dependent RNA pathway to SMA pathogenesis and evidence currently supporting the role of RNA dysfunction in the disease.

First, a clear mechanistic understanding of SMN's function in the RNA pathway of interest and a functional assay to measure this activity should be available. To date, the only molecularly defined function of SMN is in the assembly of Sm-class spliceosomal snRNPs [19, 20] and U7 snRNP [31, 33]. Second, evidence for disruption of specific SMN functions in the disease is required. SMN activity in snRNP assembly is decreased in cells from SMA patients [56] and tissue from mouse models [57, 58] where the degree of reduction correlates with disease severity [57]. Importantly, SMN-dependent deficits in RNP assembly result in reduced levels of spliceosomal snRNPs [57, 58] and U7 snRNP [33] *in vivo*. Two unexpected findings emerged from these studies. First, a remarkable difference is observed between the severe reduction in snRNP assembly activity and the relatively moderate decrease in the steady-state levels of snRNPs. Second, not all snRNPs are uniformly affected by SMN deficiency and different tissues exhibit distinct profiles of snRNP reduction. Thus, the capacity for snRNP assembly largely exceeds the minimum threshold required for snRNP synthesis *in vivo*. This threshold is likely different in distinct cell types, providing one potential explanation of how cell type specificity could result from impairment of SMN housekeeping function. Further selectivity of the effects of ubiquitous SMN deficiency relates to the surprising observation that normal motor neurons express markedly lower levels of full-length SMN mRNA from *SMN2* due to particularly inefficient exon 7 splicing compared to other cells in the spinal cord [53]. In SMA where SMN levels are reduced ubiquitously by loss of *SMN1*, motor neurons might therefore be the first to reach a threshold below which there is disruption of SMN-dependent pathways. This could be worsened through activation of a negative feedback loop by which reduced snRNP levels further decrease SMN2 exon 7 inclusion [53, 59]. Accordingly, snRNP reduction and other RNA defects appear more prominent in SMA motor neurons compared to other spinal cells in SMA mice [53]. Thus, cell type-specific differences in the efficiency of exon 7 splicing may contribute to the vulnerability of SMA motor neurons.

A disease relevant SMN-dependent RNA pathway should be required for the normal development and function of the motor system, and selective restoration of such pathway should benefit the SMA phenotype in animal models. The unexpected discovery that SMN activity is regulated in a time- and tissue-dependent manner with the highest levels found during embryonic and early postnatal development of the mouse central nervous system pointed to a differential requirement of snRNP assembly *in vivo* and a particularly high demand during neuronal development [60]. Remarkably, while ubiquitous SMN reduction in the first two postnatal weeks rapidly induces an SMA-like phenotype in mice [61], the same reduction after the spinal cord has matured–and snRNP assembly activity has reached the low, basal level that is maintained throughout life [60]–is well tolerated [61]. Thus, the requirement of SMN for the development and function of the motor system coincides temporally with the highest levels of snRNP assembly activity in the spinal cord. Furthermore, injection of purified snRNPs restored embryonic motor axon outgrowth defects in the zebrafish model of SMA [62], and allelic complementation of *SMN2* with the SMA-associated mutant SMN (A111G) improved snRNP assembly activity, restored normal

snRNP levels and corrected the disease phenotype in SMA mice [63]. Thus, increased SMN activity in snRNP assembly or restoration of snRNP levels correlate with correction of the SMA phenotype in animal models.

Defining the downstream events that result directly from disruption of SMN-dependent RNA pathways and contribute to the disease phenotype is essential for understanding SMA. Many RNA changes induced by SMN deficiency have been documented in SMA mice [33, 53, 58, 64–67]. Some of these changes can be directly ascribed to loss of SMN function in specific RNA pathways, while others are mechanistically less well understood. Furthermore, transcriptome alterations accumulate markedly in tissues of SMA mice as disease progresses [64], making it difficult to discern SMN-dependent primary events from secondary effects influenced by pathology. Recent studies focusing on the functional analysis of RNA processing events downstream of snRNP biogenesis and axonal mRNA trafficking have begun to yield candidate disease-relevant targets.

Reduced snRNP levels would be expected to cause splicing defects and mRNA expression changes. To be causally related to the disease process, selective restoration of these RNA changes should correct specific aspects of the disease phenotype in animal models. A key advance stemmed from the observation that minor snRNPs were most prominently affected by SMN deficiency in tissues of SMA mice [57, 58], leading to the hypothesis that U12 intron-containing genes could be among the disease-relevant targets. Consistent with this, SMN deficiency perturbs U12 splicing of a subset of genes in both mouse fibroblasts [65] and human SMA lymphoblasts [68]. A recent study combined the screening of all U12 intron-containing genes for regulation by SMN with functional analysis of their roles in the *Drosophila* motor circuit [65]. This approach identified the gene *Stasimon* as a U12 intron-containing splicing target of SMN that encodes a novel evolutionarily conserved transmembrane protein required for normal synaptic transmission of motor neurons. Remarkably, loss of Stasimon induces neuronal phenotypes mirroring aspects of SMN deficiency in both *Drosophila* and zebrafish, and *Stasimon* deficiency perturbs the neurotransmitter release properties of *Drosophila* motor neurons indirectly through functional disruption of other networked neurons of the motor circuit [65], analogous to SMN's requirement in this model system [54]. Furthermore, *Stasimon* restoration corrects defects in NMJ transmission and muscle growth in *Drosophila* SMN mutants as well as aberrant motor neuron development in SMN-deficient zebrafish [65]. However, *Stasimon* restoration alone did not correct all the defects induced by SMN deficiency both within and outside the *Drosophila* motor circuit [54, 65], demonstrating—perhaps not surprisingly—that disruption of this single gene does not elicit the entire SMA phenotype, which could involve defective splicing of other genes or disruption of unknown SMN-dependent events unrelated to splicing [69]. Nevertheless, the identification of *Stasimon* provided the first proof-of-concept directly linking defective splicing of a gene with essential functions in motor circuits to the phenotypic consequences of SMN deficiency in animal models. This mechanistic framework likely extends to U2 splicing as indicated by the snRNP-dependent feedback mechanisms affecting the splicing of SMN2 exon 7 in SMA motor neurons [53] and alternative splicing changes in the *neurexin2* and *UBA1* mRNAs, which have recently been implicated in the motor axon phenotype induced by SMN deficiency in zebrafish [66,

70]. Functional characterization of all disease-relevant splicing targets of SMN will build a comprehensive view of SMN-dependent splicing regulation of motor circuit function and disease.

Disruption of other SMN-dependent RNA pathways beyond splicing likely also contributes to the SMA phenotype. In the context of the proposed function(s) of SMN in axonal mRNA trafficking, *β-actin* and *cpg15/neuritin* mRNAs have been implicated in SMA due to their reduced axonal localization in SMN-deficient motor neurons and known requirement for neuronal pathfinding and synaptogenesis [45]. Overexpression of *cpg15/neuritin* has been shown to suppress motor axon outgrowth defects in SMN-deficient zebrafish embryos [46]. However, the fact that SMN deficiency decreases *cpg15/neuritin* mRNA levels in both the soma and the axon leaves open the possibility that axonal localization deficits could be secondary to other defects in mRNA metabolism. Expression of the motor neuron-specific *chondrolectin* mRNA is also reduced by SMN deficiency and its restoration improves motor axon outgrowth in the zebrafish model of SMA [71]. As the list of the genes influenced by SMN deficiency continues to grow, it will be key to define the mechanisms through which their RNA regulation is altered and the functional contribution to the SMA phenotype.

While the wide variety of animal models of SMA available have been key in revealing primary deleterious effects of SMN deficiency on the motor system, a conclusive demonstration of the disease-relevance of any SMN target requires functional testing in SMA mice as they provide the most faithful phenotypic recapitulation of the human disease. To date, none of the RNA changes implicated in SMA pathology has been evaluated functionally in mouse models. Advances in our understanding of the RNA-dependent mechanisms of SMA will come from assessment of the phenotypic effects of specific restoration of individual pathways or targets in SMA mice. This next frontier of SMA research promises to bring us closer to a complete understanding of SMA pathogenesis and possibly to the development of novel therapeutic approaches alternative to SMN upregulation.

7. Concluding remarks

Recent studies of SMN biology have highlighted not only the molecular dynamics and structural complexity by which SMN functions as an RNP assembly chaperone, but also the increasingly general role that SMN plays in post-transcriptional gene regulation. Through the identification of RNA processing defects and candidate genes whose altered expression contributes to the disease phenotype in animal models, these studies have also strengthened the molecular link between RNA dysfunction and SMA pathology. However, many aspects of SMN's role in RNA regulation have yet to be elucidated, and functional connections between SMN-dependent pathways and the pathogenesis of SMA is only beginning to emerge. Through further biochemical and functional dissection of SMN-dependent pathways and downstream targets, future efforts will focus on gaining a comprehensive knowledge of the many RBPs and cellular RNAs regulated by SMN. Identification of the cellular activities disrupted by SMN deficiency within individual components of the motor system will provide new therapeutic strategies for this currently untreatable disorder.

Finally, multiple intriguing observations have emerged from recent studies linking disruption of SMN biology to the pathogenesis of the adult-onset motor neuron disease amyotrophic lateral sclerosis (ALS) [72]. Further work is required to provide a solid molecular foundation to these early findings and to establish whether SMA and ALS—the most common forms of childhood and adult motor neuron disease, respectively—share underlying molecular defects in SMN-dependent RNA pathways. Clearly, future research on SMN biology will continue to provide novel, fundamental insights into the molecular mechanisms of RNA regulation and the basis of neurodegeneration.

Acknowledgments

We thank Neil Shneider for comments on the manuscript. We apologize to the authors whose work could not be cited owing to space limitations. Work in our laboratory is supported by grants from the US Department of Defense and NIH-NINDS.

References

1. Amaral PP, Dinger ME, Mercer TR, Mattick JS. The eukaryotic genome as an RNA machine. Science. 2008; 319:1787–9. [PubMed: 18369136]
2. Cooper TA, Wan L, Dreyfuss G. RNA and disease. Cell. 2009; 136:777–93. [PubMed: 19239895]
3. Pellizzoni L. Chaperoning ribonucleoprotein biogenesis in health and disease. EMBO Rep. 2007; 8:340–5. [PubMed: 17401408]
4. Burghes AH, Beattie CE. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nat Rev Neurosci. 2009; 10:597–609. [PubMed: 19584893]
5. Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, Viollet L, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell. 1995; 80:155–65. [PubMed: 7813012]
6. Liu Q, Dreyfuss G. A novel nuclear structure containing the survival of motor neurons protein. EMBO J. 1996; 15:3555–65. [PubMed: 8670859]
7. Battle DJ, Kasim M, Wang J, Dreyfuss G. SMN-independent subunits of the SMN complex. Identification of a small nuclear ribonucleoprotein assembly intermediate. J Biol Chem. 2007; 282:27953–9. [PubMed: 17640873]
8. Carissimi C, Saieva L, Gabanella F, Pellizzoni L. Gemin8 is required for the architecture and function of the survival motor neuron complex. J Biol Chem. 2006; 281:37009–16. [PubMed: 17023415]
9. Lorson CL, Strasswimmer J, Yao JM, Baleja JD, Hahnen E, Wirth B, et al. SMN oligomerization defect correlates with spinal muscular atrophy severity. Nat Genet. 1998; 19:63–6. [PubMed: 9590291]
10. Pellizzoni L, Charroux B, Dreyfuss G. SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins. Proc Natl Acad Sci USA. 1999; 96:11167–72. [PubMed: 10500148]
11. Martin R, Gupta K, Ninan NS, Perry K, Van Duyne GD. The survival motor neuron protein forms soluble glycine zipper oligomers. Structure. 2012; 20:1929–39. [PubMed: 23022347]
12. Tripsianes K, Madl T, Machyna M, Fessas D, Englbrecht C, Fischer U, et al. Structural basis for dimethylarginine recognition by the Tudor domains of human SMN and SPF30 proteins. Nat Struct Mol Biol. 2011; 18:1414–20. [PubMed: 22101937]
13. Kroiss M, Schultz J, Wiesner J, Chari A, Sickmann A, Fischer U. Evolution of an RNP assembly system: a minimal SMN complex facilitates formation of UsnRNPs in Drosophila melanogaster. Proc Natl Acad Sci USA. 2008; 105:10045–50. [PubMed: 18621711]
14. Fischer U, Englbrecht C, Chari A. Biogenesis of spliceosomal small nuclear ribonucleoproteins. Wiley interdisciplinary reviews RNA. 2011; 2:718–31. [PubMed: 21823231]

15. Yong J, Kasim M, Bachorik JL, Wan L, Dreyfuss G. Gemin5 delivers snRNA precursors to the SMN complex for snRNP biogenesis. Mol Cell. 2010; 38:551–62. [PubMed: 20513430]
16. Hallais M, Pontvianne F, Andersen PR, Clerici M, Lener D, Benbahouche Nel H, et al. CBC-ARS2 stimulates 3′-end maturation of multiple RNA families and favors cap-proximal processing. Nat Struct Mol Biol. 2013; 20:1358–66. [PubMed: 24270878]
17. Izumi H, McCloskey A, Shinmyozu K, Ohno M. p54nrb/NonO and PSF promote U snRNA nuclear export by accelerating its export complex assembly. Nucleic Acids Res. 2014
18. Massenet S, Pellizzoni L, Paushkin S, Mattaj IW, Dreyfuss G. The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. Mol Cell Biol. 2002; 22:6533–41. [PubMed: 12192051]
19. Meister G, Buhler D, Pillai R, Lottspeich F, Fischer U. A multiprotein complex mediates the ATP-dependent assembly of spliceosomal U snRNPs. Nat Cell Biol. 2001; 3:945–9. [PubMed: 11715014]
20. Pellizzoni L, Yong J, Dreyfuss G. Essential role for the SMN complex in the specificity of snRNP assembly. Science. 2002; 298:1775–9. [PubMed: 12459587]
21. Yong J, Golembe TJ, Battle DJ, Pellizzoni L, Dreyfuss G. snRNAs contain specific SMN-binding domains that are essential for snRNP assembly. Mol Cell Biol. 2004; 24:2747–56. [PubMed: 15024064]
22. Yong J, Pellizzoni L, Dreyfuss G. Sequence-specific interaction of U1 snRNA with the SMN complex. EMBO J. 2002; 21:1188–96. [PubMed: 11867547]
23. Battle DJ, Lau CK, Wan L, Deng H, Lotti F, Dreyfuss G. The Gemin5 protein of the SMN complex identifies snRNAs. Mol Cell. 2006; 23:273–9. [PubMed: 16857593]
24. Friesen WJ, Paushkin S, Wyce A, Massenet S, Pesiridis GS, Van Duyne G, et al. The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. Mol Cell Biol. 2001; 21:8289–300. [PubMed: 11713266]
25. Meister G, Eggert C, Buhler D, Brahms H, Kambach C, Fischer U. Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln. Curr Biol. 2001; 11:1990–4. [PubMed: 11747828]
26. Brahms H, Meheus L, de Brabandere V, Fischer U, Luhrmann R. Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B′ and the Sm-like protein LSm4, and their interaction with the SMN protein. RNA. 2001; 7:1531–42. [PubMed: 11720283]
27. Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G. SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell. 2001; 7:1111–7. [PubMed: 11389857]
28. Ishikawa H, Nobe Y, Izumikawa K, Yoshikawa H, Miyazawa N, Terukina G, et al. Identification of truncated forms of U1 snRNA reveals a novel RNA degradation pathway during snRNP biogenesis. Nucleic Acids Res. 2014; 42:2708–24. [PubMed: 24311566]
29. Marzluff WF, Wagner EJ, Duronio RJ. Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail. Nat Rev Genet. 2008; 9:843–54. [PubMed: 18927579]
30. Schumperli D, Pillai RS. The special Sm core structure of the U7 snRNP: far-reaching significance of a small nuclear ribonucleoprotein. Cell Mol Life Sci. 2004; 61:2560–70. [PubMed: 15526162]
31. Pillai RS, Grimmler M, Meister G, Will CL, Luhrmann R, Fischer U, et al. Unique Sm core structure of U7 snRNPs: assembly by a specialized SMN complex and the role of a new component, Lsm11, in histone RNA processing. Genes Dev. 2003; 17:2321–33. [PubMed: 12975319]
32. Pillai RS, Will CL, Luhrmann R, Schumperli D, Muller B. Purified U7 snRNPs lack the Sm proteins D1 and D2 but contain Lsm10, a new 14 kDa Sm D1-like protein. EMBO J. 2001; 20:5470–9. [PubMed: 11574479]
33. Tisdale S, Lotti F, Saieva L, Van Meerbeke JP, Crawford TO, Sumner CJ, et al. SMN is essential for the biogenesis of U7 small nuclear ribonucleoprotein and 3′-end formation of histone mRNAs. Cell Rep. 2013; 5:1187–95. [PubMed: 24332368]
34. Ellis RJ. Macromolecular crowding: obvious but underappreciated. Trends Biochem Sci. 2001; 26:597–604. [PubMed: 11590012]

35. Raker VA, Hartmuth K, Kastner B, Luhrmann R. Spliceosomal U snRNP core assembly: Sm proteins assemble onto an Sm site RNA nonanucleotide in a specific and thermodynamically stable manner. Mol Cell Biol. 1999; 19:6554–65. [PubMed: 10490595]
36. Palfi Z, Jae N, Preusser C, Kaminska KH, Bujnicki JM, Lee JH, et al. SMN-assisted assembly of snRNP-specific Sm cores in trypanosomes. Genes Dev. 2009; 23:1650–64. [PubMed: 19605687]
37. Chari A, Golas MM, Klingenhager M, Neuenkirchen N, Sander B, Englbrecht C, et al. An assembly chaperone collaborates with the SMN complex to generate spliceosomal SnRNPs. Cell. 2008; 135:497–509. [PubMed: 18984161]
38. Grimm C, Chari A, Pelz JP, Kuper J, Kisker C, Diederichs K, et al. Structural basis of assembly chaperone- mediated snRNP formation. Mol Cell. 2013; 49:692–703. [PubMed: 23333303]
39. Zhang R, So BR, Li P, Yong J, Glisovic T, Wan L, et al. Structure of a key intermediate of the SMN complex reveals Gemin2's crucial function in snRNP assembly. Cell. 2011; 146:384–95. [PubMed: 21816274]
40. Tharun S. Roles of eukaryotic Lsm proteins in the regulation of mRNA function. Int Rev Cell Mol Biol. 2009; 272:149–89. [PubMed: 19121818]
41. Almada AE, Wu X, Kriz AJ, Burge CB, Sharp PA. Promoter directionality is controlled by U1 snRNP and polyadenylation signals. Nature. 2013; 499:360–3. [PubMed: 23792564]
42. Kaida D, Berg MG, Younis I, Kasim M, Singh LN, Wan L, et al. U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. Nature. 2010; 468:664–8. [PubMed: 20881964]
43. Golembe TJ, Yong J, Battle DJ, Feng W, Wan L, Dreyfuss G. Lymphotropic Herpesvirus saimiri uses the SMN complex to assemble Sm cores on its small RNAs. Mol Cell Biol. 2005; 25:602–11. [PubMed: 15632062]
44. Friesen WJ, Dreyfuss G. Specific sequences of the Sm and Sm-like (Lsm) proteins mediate their interaction with the spinal muscular atrophy disease gene product (SMN). J Biol Chem. 2000; 275:26370–5. [PubMed: 10851237]
45. Fallini C, Bassell GJ, Rossoll W. Spinal muscular atrophy: the role of SMN in axonal mRNA regulation. Brain Res. 2012; 1462:81–92. [PubMed: 22330725]
46. Akten B, Kye MJ, Hao le T, Wertz MH, Singh S, Nie D, et al. Interaction of survival of motor neuron (SMN) and HuD proteins with mRNA cpg15 rescues motor neuron axonal deficits. Proc Natl Acad Sci USA. 2011; 108:10337–42. [PubMed: 21652774]
47. Fallini C, Rouanet JP, Donlin-Asp PG, Guo P, Zhang H, Singer RH, et al. Dynamics of survival of motor neuron (SMN) protein interaction with the mRNA-binding protein IMP1 facilitates its trafficking into motor neuron axons. Dev Neurobiol. 2014; 74:319–32. [PubMed: 23897586]
48. Fallini C, Zhang H, Su Y, Silani V, Singer RH, Rossoll W, et al. The survival of motor neuron (SMN) protein interacts with the mRNA-binding protein HuD and regulates localization of poly(A) mRNA in primary motor neuron axons. J Neurosci. 2011; 31:3914–25. [PubMed: 21389246]
49. Hubers L, Valderrama-Carvajal H, Laframboise J, Timbers J, Sanchez G, Cote J. HuD interacts with survival motor neuron protein and can rescue spinal muscular atrophy-like neuronal defects. Hum Mol Genet. 2011; 20:553–79. [PubMed: 21088113]
50. Tadesse H, Deschenes-Furry J, Boisvenue S, Cote J. KH-type splicing regulatory protein interacts with survival motor neuron protein and is misregulated in spinal muscular atrophy. Hum Mol Genet. 2008; 17:506–24. [PubMed: 17998247]
51. Rossoll W, Jablonka S, Andreassi C, Kroning AK, Karle K, Monani UR, et al. Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. J Cell Biol. 2003; 163:801–12. [PubMed: 14623865]
52. Zhang H, Xing L, Rossoll W, Wichterle H, Singer RH, Bassell GJ. Multiprotein complexes of the survival of motor neuron protein SMN with Gemins traffic to neuronal processes and growth cones of motor neurons. J Neurosci. 2006; 26:8622–32. [PubMed: 16914688]
53. Ruggiu M, McGovern VL, Lotti F, Saieva L, Li DK, Kariya S, et al. A role for SMN exon 7 splicing in the selective vulnerability of motor neurons in spinal muscular atrophy. Mol Cell Biol. 2012; 32:126–38. [PubMed: 22037760]
54. Imlach WL, Beck ES, Choi BJ, Lotti F, Pellizzoni L, McCabe BD. SMN Is Required for Sensory-Motor Circuit Function in Drosophila. Cell. 2012; 151:427–39. [PubMed: 23063130]

55. Mentis GZ, Blivis D, Liu W, Drobac E, Crowder ME, Kong L, et al. Early functional impairment of sensory-motor connectivity in a mouse model of spinal muscular atrophy. Neuron. 2011; 69:453–67. [PubMed: 21315257]
56. Wan L, Battle DJ, Yong J, Gubitz AK, Kolb SJ, Wang J, et al. The survival of motor neurons protein determines the capacity for snRNP assembly: biochemical deficiency in spinal muscular atrophy. Mol Cell Biol. 2005; 25:5543–51. [PubMed: 15964810]
57. Gabanella F, Butchbach ME, Saieva L, Carissimi C, Burghes AH, Pellizzoni L. Ribonucleoprotein assembly defects correlate with spinal muscular atrophy severity and preferentially affect a subset of spliceosomal snRNPs. PloS One. 2007; 2:e921. [PubMed: 17895963]
58. Zhang Z, Lotti F, Dittmar K, Younis I, Wan L, Kasim M, et al. SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. Cell. 2008; 133:585–600. [PubMed: 18485868]
59. Jodelka FM, Ebert AD, Duelli DM, Hastings ML. A feedback loop regulates splicing of the spinal muscular atrophy-modifying gene, SMN2. Hum Mol Genet. 2010; 19:4906–17. [PubMed: 20884664]
60. Gabanella F, Carissimi C, Usiello A, Pellizzoni L. The activity of the spinal muscular atrophy protein is regulated during development and cellular differentiation. Hum Mol Genet. 2005; 14:3629–42. [PubMed: 16236758]
61. Kariya S, Obis T, Garone C, Akay T, Sera F, Iwata S, et al. Requirement of enhanced Survival Motoneuron protein imposed during neuromuscular junction maturation. J Clin Invest. 2014; 124:785–800. [PubMed: 24463453]
62. Winkler C, Eggert C, Gradl D, Meister G, Giegerich M, Wedlich D, et al. Reduced U snRNP assembly causes motor axon degeneration in an animal model for spinal muscular atrophy. Genes Dev. 2005; 19:2320–30. [PubMed: 16204184]
63. Workman E, Saieva L, Carrel TL, Crawford TO, Liu D, Lutz C, et al. A SMN missense mutation complements SMN2 restoring snRNPs and rescuing SMA mice. Hum Mol Genet. 2009; 18:2215–29. [PubMed: 19329542]
64. Baumer D, Lee S, Nicholson G, Davies JL, Parkinson NJ, Murray LM, et al. Alternative splicing events are a late feature of pathology in a mouse model of spinal muscular atrophy. PLoS Genet. 2009; 5:e1000773. [PubMed: 20019802]
65. Lotti F, Imlach WL, Saieva L, Beck ES, Hao le T, Li DK, et al. An SMN-Dependent U12 Splicing Event Essential for Motor Circuit Function. Cell. 2012; 151:440–54. [PubMed: 23063131]
66. See K, Yadav P, Giegerich M, Cheong PS, Graf M, Vyas H, et al. SMN deficiency alters Nrxn2 expression and splicing in zebrafish and mouse models of spinal muscular atrophy. Hum Mol Genet. 2013
67. Zhang Z, Pinto AM, Wan L, Wang W, Berg MG, Oliva I, et al. Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. Proc Natl Acad Sci USA. 2013; 110:19348–53. [PubMed: 24191055]
68. Boulisfane N, Choleza M, Rage F, Neel H, Soret J, Bordonne R. Impaired minor tri-snRNP assembly generates differential splicing defects of U12-type introns in lymphoblasts derived from a type I SMA patient. Hum Mol Genet. 2011; 20:641–8. [PubMed: 21098506]
69. Praveen K, Wen Y, Matera AG. A Drosophila model of spinal muscular atrophy uncouples snRNP biogenesis functions of survival motor neuron from locomotion and viability defects. Cell Rep. 2012; 1:624–31. [PubMed: 22813737]
70. Wishart TM, Mutsaers CA, Riessland M, Reimer MM, Hunter G, Hannam ML, et al. Dysregulation of ubiquitin homeostasis and beta-catenin signaling promote spinal muscular atrophy. J Clin Invest. 2014
71. Sleigh JN, Barreiro-Iglesias A, Oliver PL, Biba A, Becker T, Davies KE, et al. Chondrolectin affects cell survival and neuronal outgrowth in in vitro and in vivo models of spinal muscular atrophy. Hum Mol Genet. 2014; 23:855–69. [PubMed: 24067532]
72. Achsel T, Barabino S, Cozzolino M, Carri MT. The intriguing case of motor neuron disease: ALS and SMA come closer. Biochem Soc Trans. 2013; 41:1593–7. [PubMed: 24256260]

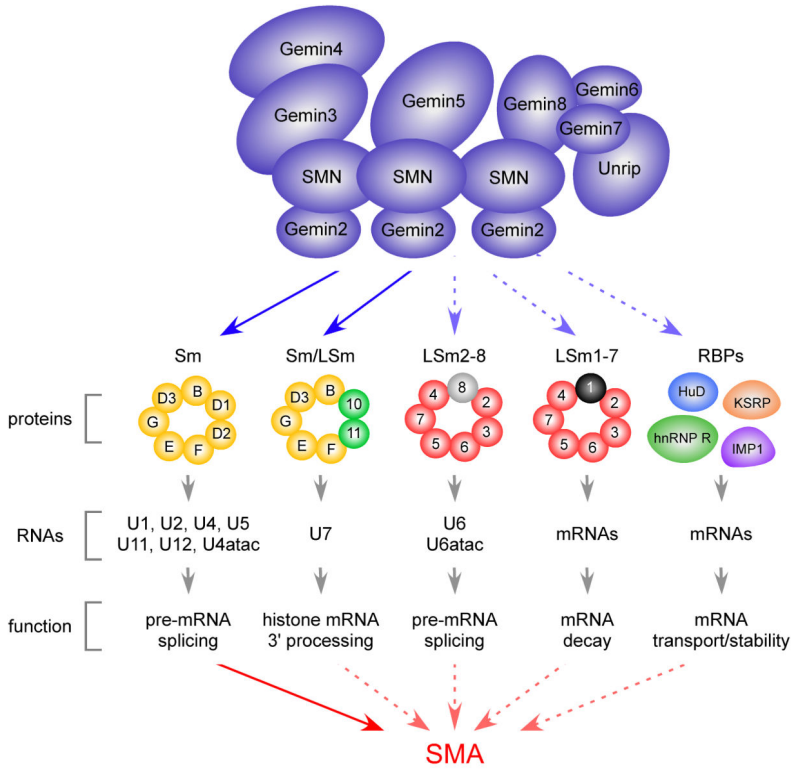

Figure 1. SMN-dependent RNP assembly pathways and their link to SMA

The SMN complex—depicted with known integral subunits according to [3]—and the protein and RNA components of each of its proposed RNP targets are shown above the RNA pathway in which they function. Solid arrows indicate connections that are established both molecularly and functionally.