HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

Biochim Biophys Acta. 2017 March ; 1860(3): 299–315. doi:10.1016/j.bbagrm.2016.12.008.

Diverse role of Survival Motor Neuron Protein

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Abstract

The multifunctional Survival Motor Neuron (SMN) protein is required for the survival of all organisms of the animal kingdom. SMN impacts various aspects of RNA metabolism through the formation and/or interaction with ribonucleoprotein (RNP) complexes. SMN regulates biogenesis of small nuclear RNPs, small nucleolar RNPs, small Cajal body-associated RNPs, signal recognition particles and telomerase. SMN also plays an important role in DNA repair, transcription, pre-mRNA splicing, histone mRNA processing, translation, selenoprotein synthesis, macromolecular trafficking, stress granule formation, cell signaling and cytoskeleton maintenance. The tissue-specific requirement of SMN is dictated by the variety and the abundance of its interacting partners. Reduced expression of SMN causes spinal muscular atrophy (SMA), a leading genetic cause of infant mortality. SMA displays a broad spectrum ranging from embryonic lethality to an adult onset. Aberrant expression and/or localization of SMN has also been associated with male infertility, inclusion body myositis, amyotrophic lateral sclerosis and osteoarthritis. This review provides a summary of various SMN functions with implications to a better understanding of SMA and other pathological conditions.

Keywords

Spinal muscular atrophy; SMA; Survival Motor Neuron; SMN; splicing; snRNP biogenesis; snoRNP biogenesis; SBP2; telomerase; TERC; TERT; TMG; transcription; splicing; DNA repair; selenoprotein; signal recognition particle; Cajal body; Gem

1. Introduction

Survival Motor Neuron (SMN) is a multifunctional protein expressed in all cell types of the animal kingdom. The importance of SMN in humans was first realized when deletions or mutations in the *SMN1* gene were found to cause Spinal Muscular Atrophy (SMA), the leading genetic disease of children and infants [1–4]. Owing to duplication and inversion, humans carry an additional centromeric copy of the *SMN* gene, *SMN2* [2]. *SMN1* codes for SMN, while *SMN2* primarily produces the truncated protein isoform (SMNΔ7) due to

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Disclosures and competing interests: ISS-N1 target (US patent # 7,838,657) mentioned in this review was discovered in the Singh lab at UMASS Medical School (Worcester, MA, USA). Inventors, including RNS, NNS and UMASS Medical School, are currently benefiting from licensing of ISS-N1 target (US patent # 7,838,657) to IONIS Pharmaceuticals (formerly ISIS Pharmaceuticals), Carlsbad, CA, USA. SpinrazaTM (synonyms: Nusinersen, IONIS-SMNRX, ISISSMNRX) is an ISS-N1-targeting oligonucleotide that has been recently approved by United States Food and Drug Administration (FDA) as the first drug for the treatment of SMA.

predominant skipping of exon 7. Thus, *SMN2* fails to fully compensate for the loss of *SMN1* [5,6]. Although SMNΔ7 is less stable and only partially functional [7–9], overexpression of this isoform reduces the disease severity in a mouse model of SMA [10]. Mice carry a single *Smn* gene. Deletion of *Smn* gene is embryonic lethal [11]; however, introduction of human *SMN2* rescues the embryonic lethality [12]. The presence of a single copy of *SMN2* in mice lacking *Smn* gene produces a phenotype resembling that of severe SMA [12]. A higher copy number of *SMN2* is associated with reduced disease severity [13,14]. Several protein factors, including Plastin3, NAIP, H4N4, IGF1, ZPR1 and UBA1, have been suggested as modifiers of SMA severity [15–19]. However, none of these factors have the ability to fully compensate for the loss of SMN functions.

Human SMN contains 294 amino acids and harbors multiple domains, including N-terminal Gemin2- and nucleic acid-binding domains, a central Tudor domain and C-terminal proline-rich and YG domains (Fig. 1). Mutations in all domains have been linked to SMA [28], suggesting that the overall structure of the protein is critical for its functions in humans. SMN localizes to both nuclear and cytosolic compartments. In particular, SMN plays an essential role in the formation of nuclear gems that share several components with the Cajal (coiled) bodies (CBs) [29–31]. CBs are dynamic nuclear structures that serve as the storehouse and/or maturation site for the ribonucleoprotein (RNP) complexes, including small nuclear RNPs (snRNPs), small nucleolar RNPs (snoRNPs), small CB-specific RNPs (scaRNPs) and telomerase RNP complexes [32]. SMN interacts with coilin, a signature protein of CBs [33]. The interaction between SMN and coilin is facilitated by WRAP53, a WD40 domain-containing protein, which is also essential for the localization of the SMN complex to CBs [34]. The relative abundance of SMN in various subcellular compartments is dependent upon the cell type [35]. Within the cytosol, SMN localizes to sarcomeric Z-discs, microtubules, the Golgi network and cytosolic stress granules (SGs) [36–46].

Alternative splicing of *SMN1* and *SMN2* generates several transcripts under normal and oxidative-stress conditions [47–50]. One of the *SMN* isoforms is produced by retention of intron 3. It codes for axonal-SMN (a-SMN) that plays a developmental role in mammalian brain [47]. a-SMN promotes axon growth, stimulates cell motility and regulates expression of chemokines (CCL2, CCL7) and insulin-like growth factor-1 [51]. The *a-SMN* transcripts are generally not detected in adult tissues likely due to their degradation by the nonsense-mediated decay (NMD) pathway. Another SMN protein isoform, SMN6B, is generated by exonization of an Alu-like sequence located within intron 6 (Fig. 1) [50]. While the *SMN6B* splice isoform is subject to NMD, SMN6B protein was shown to be more stable than SMNΔ7 [50]. The functions of SMN6B as well as SMN isoforms generated by skipping of exons 5 and/or 3 remain unknown.

In addition to SMA, the involvement of SMN has also been shown in other pathological conditions. For instance, the role of SMN has been implicated in inclusion body myositis, amyotrophic lateral sclerosis (ALS) and osteoarthritis [52–54]. Supporting a role of SMN in mammalian testicular development and male fertility, its levels in testis are very high compared to other organs and tissues due in part to the predominant inclusion of exon 7 during *SMN2* pre-mRNA splicing [55,56]. Consistently, under the condition of decreased SMN levels, male mice display defective testicular development, impaired spermatogenesis

and reduced fertility [57]. Interestingly, an aberrant high SMN expression was recorded in osteoarthritis cartilage compared to the normal cartilage [54]. However, it remains to be seen if the high SMN expression in osteoarthritis cartilage is a cause or effect of osteoarthritis.

SMA happens to be a unique genetic disease, since *SMN2* copy of the gene is almost universally present in patients. Hence, *SMN2* is considered to be the most promising therapeutic target. Currently, compounds that enhance *SMN2* transcription, correct *SMN2* exon 7 splicing, increase stability of SMN and/or SMNΔ7 proteins and allow stop codon read through of *SMN2Δ7* transcripts, are being considered as potential candidates for SMA therapy [58]. An antisense-based drug targeting intronic splicing silencer N1 (ISS-N1) we discovered in 2006 has just completed phase 3 clinical trials and is likely to become the first FDA-approved drug for SMA [28,59–62]. Many recent reviews describe the role of SMN in neurodegeneration and the progress toward SMA therapy [63–67]. Similarly, several reports summarize the current knowledge of cis-elements and transacting factors that are involved in regulation of *SMN* transcription and splicing [68–71]. The purpose of this review is to focus on the diverse nature of SMN functions and discuss how reduced levels of SMN might differentially affect a variety of human tissues. Based on the presence of sequence/structural motifs and the nature of SMN interactions, it is obvious that SMN functions have continued to evolve and diversify. We will describe how lessons learnt from the employment of various model systems and profiling studies are improving our understanding of SMN functions. Given a broad spectrum of SMA phenotype and related diseases, we are tempted to speculate that SMN has multiple housekeeping functions that are differently regulated in various cell types.

2. Domain Organization

Alignment of SMN amino acid sequences across several species shows its remarkable conservation in higher vertebrates (Fig. 2). Three stretches of more than fifty conserved residues of vertebrate SMN are located at the N-terminus, central region and C-terminus (Fig. 2). All of these regions are known to have interacting partners (Fig. 1). The nucleic acid-binding domain coded by exons 2A and 2B is conserved and overlaps with the binding site of the SMN-Interacting Protein 1 (SIP1), also known as Gemin2 [72–74]. The core complex formed by SMN-Gemin2 appears to be central to most functions of SMN in vertebrates, including snRNP assembly, DNA recombination, signal recognition particle biogenesis and translation regulation [20,75–77]. The domain encoded by exon 2 also interacts with p53, a tumor suppressor protein and transcription regulator [78]. Exon 3 of *SMN* codes for a Tudor domain that is involved in interactions with proteins carrying RGG/RG motifs, which are symmetrically dimethylated [79–81]. The examples of these proteins include but are not limited to GAR1, Fibrillarin, hnRNP Q, hnRNP R, hnRNP U, Ewing's Sarcoma Protein (EWS), Fragile X Mental Retardation Protein (FMRP), Fused in Sarcoma (FUS), Sm proteins, Histone 3 and the carboxy terminal domain (CTD) of RNA Polymerase II (pol II) [29,82–92]. Downstream of the Tudor domain, SMN contains a proline-rich sequence (Fig. 1). This sequence interacts with Profilins, a family of small proteins that control the actin dynamics in the cell [93]. The last sixteen amino acids (coded by exon 7) together with the upstream YG box (coded by exon 6) facilitate self-oligomerization that appears to be critical for stability and subcellular localization of SMN

[9,94,95]. The C-terminal sequences of SMN, including YG box, are also involved in the interaction with Gemin3 (a dead-box helicase), ZPR1 (a zinc-finger protein) and SIN3A (a transcription co-repressor) [96–98]. The loss of amino acids coded by exon 7 has been shown to abrogate SMN interaction with Trimethylguanosine Synthase 1 (TGS1), which catalyzes the formation of 2,2,7-trimethylguanosine (TMG) cap structure at the 5′-end of the snRNAs, snoRNAs and a subpopulation of mRNAs [99,100]. The QNQKE motif present within sequences coded by exon 7 serves as a nuclear export signal [35]. Zebrafish Smn with mutations in QNQKE motif retains the snRNP assembly function but fails to rescue motor axon defects [101]. Skipping of exon 7 adds a four-amino acid motif, EMLA, coded by exon 8. EMLA serves as a degradation signal for SMNΔ7, which explains its decreased stability [9]. Compounds that allow read through of the stop codon in exon 8 and cause a few amino acids being added downstream of EMLA, increase the protein stability and show therapeutic efficacy in mouse models of SMA [102,103].

The C-terminal YG box of SMN is the most conserved motif from yeast to humans (Fig. 2). The YG box of yeast SMN is needed for cell viability but is dispensable for interactions with Sm proteins and self-oligomerization [104]. Interestingly, the genome of *Arabidopsis thaliana* lacks a true ortholog of SMN [105]. These observations support a point of view that in lower eukaryotes and plants other proteins perform SMN-like functions. Despite conservation of several SMN motifs among vertebrates, noticeable differences do exist at the N- and C-termini (Fig. 2). In particular, the N-terminus of SMN appears to be specific to mammals; it harbors binding sites for several critical interacting partners (Fig. 1). Consistently, SMNΔN27, a SMN mutant lacking 27 N-terminal amino acids, displays a dominant negative effect on various SMN functions, including splicing, snRNP reorganization, telomerase activity and hyper methylation by TGS1 activity [99,106,107]. The absence of the mammalian-specific N-terminal sequences in lower vertebrates suggests that during evolution mammalian SMN has undergone drastic changes in its structure and functions. Additionally, in comparison to mammalian SMN, the polyproline region is substantially shorter in non-mammal vertebrates; it is completely absent in *Drosophila melanogaster* and *Caenorhabditis elegans* (Fig. 2). There appears to be further addition to the structure/function of primate SMN due in part to the inclusion of a coding exon derived from an Alu element [50].

3. Role of SMN in RNA metabolism

SMN controls various aspects of the RNA metabolism, including but not limited to transcription [90], pre-mRNA splicing [106], snRNP assembly [20,72,108–115], the 3′ end of histone mRNA processing [116,117], snoRNP assembly [82,118,119], telomerase activity [119], SG formation [120], translation [121,122], signal recognition particle (SRP) biogenesis [123] and mRNA trafficking [124–130] (Fig. 3). Here we provide a brief description of the RNA metabolism pathways that are impacted by the low SMN levels.

3.1. Spliceosomal snRNP assembly

Spliceosomal snRNP assembly is the most studied function of SMN thanks to the pioneering work from the Dreyfuss laboratory [20,72,108–112]. The action of SMN in snRNP assembly

is executed by a large SMN complex comprised of SMN, Gemin proteins (Gemins2–8) and Unrip [113–115]. The SMN complex is quite stable, since most of its components remain tightly associated even at very high salt concentration (500 nM NaCl) [131]. A spliceosomal snRNP is comprised of a snRNA and a heptameric ring of Sm proteins (B/B′, D1, D2, D3, E, F, and G) [132]. The function of the SMN complex in ATP-dependent snRNP assembly has been demonstrated in vitro [109]. The in vivo process of snRNP biogenesis involves multiple distinct steps in the nucleus and cytosol. In the nucleus, a snRNA is transcribed by pol II and the newly synthesized snRNA undergoes co-transcriptional processing in which a 7-methyl guanosine cap (m^7G-cap) is added to the 5′ end and the 3′ end is cleaved, generating a pre-snRNA [132]. Then a multiprotein export complex comprised of Cap-Binding Proteins (CBP20 and CBP80), Phosphorylated Adaptor for RNA Export (PHAX), Exportin 1 (Xpo1) and RanGTP is assembled on this pre-snRNA to export it to the cytosol [115]. Additional factors, including ARS2, p54nrb/NonO and PSF also participate in this process [133,134].

Once in the cytosol, the export complex is disassembled and the pre-snRNA undergoes further processing by SMN complex, such as loading of the heptameric Sm ring to the pre-snRNA. Several steps ensure the specificity of the process. For example, the Protein Arginine Methyltransferase 5 (PRMT5) complex performs symmetrical dimethylation of a subset of Sm proteins, which leads to their tighter interactions with SMN [115]. Further, Gemin5 of SMN complex recognizes specific sites on the pre-snRNA for loading of the heptameric Sm ring [110]. Recently it has been shown that U1-70K, a component of U1 snRNP, can substitute the functions of Gemin5 in snRNP assembly [112]. After loading of the Sm ring, the pre-snRNA is subjected to hypermethylation of its m^7G-cap by TGS1 to acquire the TMG cap structure [135]. At this stage, the pre-snRNA also undergoes the 3′ end trimming [132]. A direct interaction between SMN and TGS1 appears to be essential for the formation of the TMG cap structure on pre-snRNAs [99,136]. Still bound to SMN complex, the newly processed snRNP is imported back into the nucleus. The TMG cap and the Sm core serve as the nuclear localization signal [137–139]. A direct interaction between SMN and Importin-β facilitated by WRAP53 has also been implicated in the nuclear import of snRNPs [34,95]. Once in the nucleus, the snRNA goes through final maturation in CBs. In particular, a handful of nucleotides of snRNAs are pseudouridylated or 2′-O-methylated. Interactions between SMN and WRAP53 appear to play an important role in the targeting of snRNPs as well as other RNP complexes, such as snoRNPs and telomerase, to CBs [140].

Consistent with the critical role of SMN in snRNP assembly, SMN deficiency causes widespread defects in splicing [141–145]. It has been argued that the splicing of minor introns in particular is affected in SMA [146]. There is evidence to suggest that some of the effects on alternative splicing are indirect, since levels of factors that are involved in splicing could also be affected by downregulation of SMN [122]. Overall, the mechanism by which SMN deficiency triggers aberrant splicing of specific introns remains to be understood.

3.2. Biogenesis of snoRNPs

snoRNPs belong to a class of RNP complexes that perform posttranscriptional modifications of non-coding RNAs, such as ribosomal RNAs (rRNAs) and snRNAs [147]. A typical

snoRNP encompasses a small guide RNA (snoRNA), which defines the site of posttranscriptional modifications [148], and specific protein factors. Based on the sequence and structural motifs, snoRNAs fall into two broad categories, i.e. H/ACA box and C/D box. While the H/ACA box snoRNAs guide pseudouridylation, C/D box snoRNAs guide 2′-O-methylation. A recent review describes various types of snoRNAs and their potential targets [149]. Both types of snoRNAs are defined by specific secondary structures. In the case of the H/ACA box snoRNAs, two hairpin structures are joined by a single-stranded region carrying the H box (ANANNA, where N = G, U, C, A) and the 3′-end region carrying the ACA box (AYA, where Y = C or U) motifs. The 5′-end of the C/D box snoRNA contains the C box motif (RUGAUGA, where R is purine), whereas, the D box motif (CUGA) is located near the 3′-end. The secondary structure of a snoRNA brings C and D box motifs in close proximity due to a stem formed by the base pairing of the 5′-end sequences with the 3′-end sequences [149]. The defined secondary structures of snoRNAs are necessary for the interaction with the target RNAs as well as with protein components of snoRNPs.

Different sets of core proteins and additional auxiliary factors interact with different classes of snoRNAs. The core protein components of H/ACA box snoRNAs include Dyskerin, GAR1, NHP2 and NOP10. Dyskerin carries the essential catalytic function of pseudouridylation performed by H/ACA box snoRNPs. The core protein components of C/D box snoRNP include 15.5K, NOP56, NOP58 and fibrillarin [148]. Fibrillarin carries the essential methyl transferase activity of C/D box snoRNPs [147]. Supporting its involvement in snoRNP biogenesis and/or function, SMN was shown to interact with GAR1 and Fibrillarin [82,118]. Consistent with these findings, SMN and Fibrillarin co-localize within dense fibrillary components of nucleoli of HeLa cells [82]. As per several other studies, a substantially greater abundance of SMN is observed in nucleoli of primary tissues than of cultured cells [150–152]. Interestingly, SMN also interacts with NAF1, a non-snoRNP protein responsible for the assembly of the H/ACA box class of snoRNPs [119]. However, it remains to be seen if the assembly of the H/ACA box snoRNPs is differentially impacted by the low levels of SMN.

A subset of snoRNPs referred to as scaRNPs (small CB-specific RNPs) localizes to CBs; they are mainly involved in snRNA modifications [153]. A CAB box (UGAG) motif within the hairpin loop of H/ACA box of scaRNAs serves as the guide sequence for the localization of scaRNPs to CBs [154]. Experiments in *D. melanogaster* have shown that WDR79, a homolog of human WRAP53, interacts with the CAB box and transports CAB box-containing scaRNPs to CBs [155]. In case of the C/D box scaRNAs, a long UG dinucleotide repeat serves as the CB-targeting sequence [156]. However, factors involved in the transporting of C/D box scaRNAs to CBs have not yet been identified. SMA patient cells show disruption of CB formation as well as decreased localization of snoRNP/scaRNP chaperone Nopp140 to CBs [34,157]. Depletion of SMN leads to similar consequences [34]. It is likely that the interaction of SMN with the components of scaRNPs coupled with the interaction of SMN with WRAP53 and Coilin drives localization of scaRNPs to CBs.

3.3. Biogenesis of telomerase

Telomerase is a multi-component RNP complex that catalyzes replication of chromosomal ends. Subunits of a human telomerase include a RNA component (TERC), a reverse transcriptase (TERT) and proteins associated with H/ACA box scaRNAs [158]. Human TERC is transcribed by pol II and accumulates in cells as a 451 nt-long RNA after being processed from a longer transcript [159]. The secondary structure of TERC provides the context for RNA-protein interactions as well as for defining the boundaries of the template for TERT [158]. In particular, the 5′-end of TERC folds into a pseudoknot structure and encompasses the template and the binding site of TERT. A conserved region (CR4-CR5) in the middle of TERC also interacts with TERT. The 3′-end of TERC folds into an H/ACA box scaRNA-like structure and interacts with NHP2, NOP10, GAR1, Dyskerin and WRAP53/TCAB1 [158]. The involvement of SMN in telomerase-associated functions has been proposed based on the findings that SMN interacts with multiple components of telomerase, including GAR1, TERT, Dyskerin and WRAP53 [82,107,119]. One of the likely consequences of the above-mentioned interactions of SMN is the transport of telomerase to CBs. Considering CBs associate with telomeres during S-phase [160], SMN is likely to have an influence on the maintenance of the chromosome telomeres. SMN may also facilitate the de novo assembly of telomerase, since it also interacts with NAF1, a factor responsible for the de novo assembly of the H/ACA box class of snoRNPs [119]. SMNΔN27, the dominant negative isoform of SMN, inhibits the telomerase reaction in vitro [107], suggesting that SMN may have a direct effect on the catalytic function of telomerase.

3.4. The 3′ end processing of histone mRNAs

Histone mRNAs require special 3′ end processing, since they are not polyadenylated, and U7 snRNP plays an essential role in this process [161]. The 3′ end of histone mRNAs contains a stem-loop structure followed by a cleavage site. U7 snRNP is recruited downstream of the cleavage site and in conjunction with a stem-loop-binding protein and other factors, facilitates the cleavage at the 3′ end of histone mRNAs [161]. Except for a few differences, the overall architecture of U7 snRNP resembles those of spliceosomal snRNPs. Instead of SmD1 and SmD2 found in spliceosomal snRNPs, the heptameric ring of U7 snRNP harbors Sm-like proteins Lsm10 and Lsm11 [116,162]. While the role of the SMN complex in U7 snRNP assembly is similar to that of spliceosomal snRNP assembly, the composition of the SMN complex involved in the U7 snRNP biogenesis is proposed to be distinct [116,162]. Consistent with the critical role of SMN in U7 snRNP assembly and histone metabolism, SMN deficiency causes accumulation of U7 snRNA and the defective processing of the 3′ end of histone mRNAs [117].

3.5. Pre-mRNA splicing

Pre-mRNA splicing is an essential process by which spliceosome removes introns in eukaryotes. In addition to the core components of spliceosome, several auxiliary factors are also involved in pre-mRNA splicing (see refs. in 48). Independent of its role in snRNP biogenesis, there is evidence to support the role of SMN as an auxiliary factor in pre-mRNA splicing. For instance, an early in vitro study employing chicken δ-crystallin pre-mRNA showed suppression of a splicing reaction when nuclear extract was pre-incubated with a

SMNΔN27 [106]. This suppression was not observed when pre-incubation was performed with SMN, suggesting that the N-terminal sequences of SMN are critical for the assembly of the spliceosome [106]. A splicing reaction involves formation of an early commitment complex or E complex that brings the 5′- and 3′-splice sites in close proximity. Composition of the E complex as well as subsequent steps might vary depending on the sequence of the pre-mRNA and the cell type. A recent study analyzed the composition of the E complex assembled on MINX, an adenovirus derived sequence, and identified several components of the SMN complex, including SMN [163]. However, the mechanism by which SMN promotes the formation of the E complex remains unknown. SMN may affect splicing of specific exons by interacting with other splicing factors. Supporting this argument, SMN-interacting proteins hnRNP U, hnRNP R and FUS were also detected in the E complex assembled on MINX [163]. RNA helicases modulate pre-mRNA splicing by unwinding RNA structures of pre-mRNAs [164]. Several structural elements have been implicated in splicing regulation of *SMN* as well as other genes [165–171]. In vivo selection of the entire exon also supports the role of RNA structure in regulation of *SMN* exon 7 splicing [172,173]. In addition, different types of antisense oligonucleotides annealing to various positions within *SMN2* pre-mRNA have been shown to promote exon 7 inclusion [174–183]. The stimulatory effects of these antisense oligonucleotides could be due at least in part to perturbations in the local RNA structures. Considering SMN associates with RNA helicases, including Gemin3, DDX1, DDX3 and DDX5 [92,96], it is likely that SMN modulates its own splicing as well as splicing of other transcripts through helicase interactions. Splicing is coupled to transcription and several splicing factors are recruited during this process [184,185]. SMN may indirectly affect transcription-coupled splicing regulation through its interacting partners, such as FUS and helicases that associate with pol II [186]. Since SMN controls pausing at the transcription termination site [90], it may also modulate splicing of last introns by recruiting splicing factors during transcription termination.

3.6. Transcription

Transcription is a multistep process consisting of initiation, elongation and termination. The role of SMN in one or more of these steps could be envisioned based on the finding that SMN directly interacts with the CTD of pol II [187]. Independent of pol II interaction, SMN also binds to transcription factors and chromatin remodeling complexes [78,98,188]. For example, SMN interacts with papillomavirus-encoded transcription factor E2 and enhances E2-dependent transcriptional activation [188]. SMN also binds to p53, a transcription factor with distinct nuclear localization, DNA-binding and transactivation domains [78]. Interestingly, SMN-p53 complex localizes to CBs, which are maintained by WRAP53, an SMN-interacting protein generated from the antisense transcript of p53 gene [78,119]. SMN binding to E2 and p53 suggests its role in transcription initiation. Supporting its participation in chromatin-associated transcription regulation, SMN interacts with SIN3A, a transcription co-repressor [98]. SIN3A serves as a master scaffold for histone deacetylases (HDACs) and other proteins that modulate chromatin structure and transcription [189]. Transcription elongation requires directionality that is decided by the prompt interaction of U1 snRNP with the nascent transcript while it is still attached to transcribing pol II [190]. Therefore, SMN may also affect transcription elongation indirectly by controlling the rate of biogenesis

of U1 snRNP that happens to be the most abundant snRNP in the nucleus. Further, pol II creates R-loops in transcription termination regions; these R-loops must be resolved for the nascent transcripts to be released from the DNA template. Supporting the role of SMN in transcription termination, SMN interacts with Senataxin, a putative DNA/RNA helicase, which is involved in the resolution of R-loops [191]. More recently, a role for SMN in resolution of R-loops and transcription termination has been established through its direct interaction with the symmetrically dimethylated residues of the CTD of pol II [90].

3.7. RNA trafficking

SMN harbors a nucleic acid-binding domain and has preference for homopolymeric G residues in vitro [73,74]. An early study suggested the role of SMN in trafficking of *β-Actin* mRNAs in neuronal processes and growth cones [40]. SMN assembled on *β-Actin* mRNA was shown to also interact with hnRNP R, an RNA-binding protein [40]. Other RNA-binding proteins implicated in mRNA trafficking in motor neurons, such as FMRP, HuD, Insulin-Like Growth Factor mRNA-Binding Protein 1 (IMP1), KH-Type Splicing Regulatory Protein (KSRP) and hnRNP Q, have been shown to interact with SMN as well [87,124–129]. HuD, a member of the Hu family of proteins, is expressed only in neuronal cells; it interacts with a wide variety of RNA sequence motifs [192,193]. HuD and IMP1, the mammalian homolog of Zip-Code Binding Protein 1 (ZBP1), have been shown to interact with overlapping motifs within the 3′UTR of the *β-Actin* mRNA [193]. While HuD shows some preference for U-rich sequence, ZBP1 binds to the ACACCC motif in the structured region [193]. *Candidate Plasticity-Related Gene 15* (*cpg15*) mRNA is another target of HuD. It has been proposed that SMN facilitates trafficking of HuD-bound *cpg15* mRNA to the axonic terminals for local translation [127]. More recently, the SMN/HuD/IMP1 complex has been implicated in the transport of *Growth-Associated Protein 43* (*Gap43*) in motor neurons [130]. Consistently, overexpression of HuD and IMP was found to rescue the axon outgrowth defects in cultured primary motor neurons derived from a severe mouse model of SMA [130].

Based on the broad sequence specificity of RNA-binding proteins that interact with SMN, SMN may be involved in trafficking of a large number of mRNAs in motor neurons. Indeed, a transcriptome-wide study employing differentiated NSC-34 motor-neuron-like cells identified more than 200 mRNAs, including *Smn*, as potential targets of SMN [128]. The SMN-interacting protein hnRNP Q was found to be one of the major components of the SMN complex associated with these mRNAs. However, the presence of other RNA-binding proteins, including HuD, IMP1 and KSRP was not verified. Of note, HuR, a widely expressed member of the Hu family of proteins, has been shown to stabilize *SMN* mRNA by interacting with its 3′UTR [194]. However, it remains to be seen if the interaction of HuR with the 3′UTR of *SMN* mRNA is modulated by SMN levels and is critical for the intracellular trafficking of *SMN* mRNA.

3.8. Biogenesis of the Signal Recognition Particle

The signal recognition particle (SRP) is a ubiquitously expressed cytosolic RNP complex involved in the localization of specific proteins [195,196]. In particular, SRP interacts with the newly synthesized hydrophobic N-terminus of proteins that serves as the signal for the

transport of these proteins to the endoplasmic reticulum. SRP is comprised of six proteins (SRP9, 14, 19, 54, 68, and 72) and a single RNA molecule, 7S RNA [195,196]. The secondary structure of 7S RNA can be divided into three distinct folds in which a large S domain and a small Alu domain flank the central helix. Supporting the role of SMN in SRP biogenesis, the purified SMN complex was found to interact with 7S RNA in vitro [123]. It was further shown that Gemin5 directly binds the S-domain and that the SMN complex is required for the assembly of the SRP54 protein onto 7S RNA [123]. In addition, the level of 7S RNA was significantly reduced in the spinal cord of SMA mice, indicating a requirement for high levels of SMN for proper expression of functional SRPs [123].

3.9. Translation

SMN has been implicated in translation regulation of *Protein Arginine Methyltransferase 4* (*PRMT4*), also known as *Coactivator Associated Arginine Methyltransferase 1* (*CARM1*) [121]. CARM1 is a multifunctional protein that affects transcription, splicing and autophagy [122,197–199]. Downregulation of SMN increases the level of CARM1 [122]. Consistently, CARM1 is upregulated in tissues from SMA mouse models as well as in SMA type I patient cells [121]. Increased expression of CARM1 has been shown to cause an aberrant increase in inclusion of exon 2 of the *Ubiquitin-Specific Protease-Like 1* (*USPL1*) gene that codes for a SUMO isopeptidase [122]. The exon 2-containing transcript of *USPL1* is also upregulated in mouse models of SMA as well as in SMA type I patient cells [122,141,142,200]. These findings support a point of view that the aberrant splicing of *USPL1* exon 2 in SMA is the consequence of upregulation of CARM1. CARM1 interacts with UPF1, a key component of the NMD pathway, and affects the fate of a subset of NMD targets [122]. However, NMD has been ruled out as a possible mechanism by which exon 2-containing transcripts of *USPL1* are enriched in SMA [122]. Interestingly, inclusion of *USPL1* exon 2 was found to be more pronounced in muscle than in spinal cord of SMA mice [141]. It is not known if the tissue-specific difference in exon 2-containing transcripts of *USPL1* is due to a corresponding difference in the CARM1 levels. SMN may also have an indirect role in translation repression through the RNA interference pathway, since several microRNAs, including miR-9, miR-183, miR-206, miR-132 and miR-431 are aberrantly expressed in SMA [201–205]. Some of these microRNAs have been suggested to be potential targets for SMA therapy [204,205].

3.10. Selenoprotein synthesis

Humans code for 25 selenoproteins that incorporate selenocysteine (Sec), the 21st naturally occurring amino acid, into their primary structure [206]. Incorporation of Sec into selenoproteins occurs due to recoding of a stop codon, UGA, when a Sec insertion sequence (SECIS) is present downstream [206,207]. SECIS-Binding Protein 2 (SBP2), Sec-Specific Translation Elongation Factor (EF^{sec}) and tRNA^{sec} play an important role in Sec incorporation into selenoproteins [206]. Recently, mRNAs of a subgroup of selenoproteins were also shown to acquire a TMG cap structure through a TGS1-catalyzed reaction in the cytosol [100]. The results of this study revealed a RNA-independent interaction among SMN, SBP2 and TGS1 [100]. Generally, the TMG cap is associated with nuclear retention. However, selenoprotein mRNAs that included a TMG cap were found to be retained in the cytosol and were actively translated [100]. These findings point to a novel mechanism by

which SMN regulates initiation of translation of a subset of selenoprotein mRNAs. Interestingly, mRNA of selenoprotein SepW1 colocalizes with SMN-associated RNP complexes in the neurites of the mouse motor-neuron-like NSC-34 cells [128]. These observations support a specific role of SMN in trafficking and translation of *SepW1* mRNA in motor neurons. Several selenoproteins possess antioxidant functions that appear to be compromised in a number of diseases [207]. However, the consequences of low SMN levels on the synthesis of various selenoproteins in different tissues have not yet been assessed. Human *SBP2* generates multiple alternatively spliced transcripts under normal and oxidative stress conditions [49,208,209]. Future studies will determine if various *SBP2* isoforms interact with the SMN-TGS1 complex differently with the implications for the trafficking and/or translation of specific selenoprotein mRNAs.

3.11. Stress Granule (SG) Formation

SGs are dynamic cytosolic storage hubs for mRNAs, translation initiation of which are stalled during stress [210]. SGs share several features with processing bodies (PBs) that are cytosolic triage centers of mRNAs [211]. The distinguishing characteristics of SGs are the presence of the translation initiation machinery, whereas PBs are defined by the presence of the mRNA decay machinery. The repertoire of factors present within SGs is large, varied and includes RNA-binding proteins, metabolic enzymes, signaling factors, mRNAs and microRNAs [210,211]. SG formation is dysregulated in various pathological conditions, including cancer and neurodegeneration [211–213]. SMN localizes to SGs and SMN deficiency reduces the ability of cells to form SGs leading to the cell sensitization to stress [46,120]. Isolated SMN domains coded by exons 2A+2B or exons 4–7 are able to form small SGs [120]. However, the Tudor domain coded by exon 3 along with adjacent domains coded by exons 4–7 appear to be essential for the formation of large SGs [120]. Consistent with these results, FMRP that interacts with SMN through the Tudor domain has been identified as a component of SGs [214]. The fact that the nucleic-acid-binding domain coded by exon 2B of *SMN* is sufficient to form small SGs supports that SMN might transport mRNAs to SGs. Cellular levels of SMN are governed by TIA1 and several other factors that regulate *SMN* exon 7 splicing [215]. Similar to SMN, TIA1 is also a component of SGs [211]. Sequestration of TIA1 in SGs is likely to reduce its nuclear availability and consequently induce skipping of *SMN2* exon 7. Future studies will determine if the formation of SGs is a mechanism by which SMN senses its own levels so that appropriate amount of SMN could be generated and delivered to various subcellular compartments.

4. DNA recombination and repair

Eukaryotic cells employ homologous DNA recombination to effectively repair DNA in diploid cells as well as to exchange genetic material between homologous chromosomes during meiosis [216–218]. Among the factors involved in homologous recombination, RAD51, a eukaryotic recombinase, plays an essential role [219]. In particular, RAD51 forms a filament on the single-stranded DNA. The formation of the RAD51 filament is essential for the homology search and strand exchange steps of the homologous recombination [219]. The homologous pairing and the strand exchange mediated by RAD51 could be recapitulated in vitro [220]. Supporting a direct role of SMN in homologous recombination,

RAD51 has been shown to interact with GEMIN2 and the SMN-GEMIN2 complex [221,222]. It has also been demonstrated that the interaction of RAD51 with the SMN-GEMIN2 complex enhances the RAD51-mediated homologous pairing and strand exchange reaction in vitro (Fig. 4) [221,222]. These findings gain additional significance in light of reports supporting a relationship between DNA repair and R-loop-mediated genome stability [223]. Since SMN is involved in resolution of R-loop [90], it is possible that the positioning of SMN at the R-loop facilitates DNA repair.

In addition, SMN is recruited through an interaction with histone H3 to centromeres in the presence of DNA damage and it participates in the induced Centromeric Damage Response (iCDR) [89]. SMN also plays an indirect role in DNA damage response due to a dependence of histone H2AX expression on SMN levels [117]. Further, DNA damage is repaired during transcription elongation through a process called transcription-coupled repair (TCR) [224]. Given the interaction of SMN with pol II [90], it is likely that SMN modulates the process of TCR. Consistent with the role of SMN in DNA repair, DNA damage has been reported as one of the early symptoms in the skeletal muscles of a mouse model of SMA [225]; this finding suggests a direct involvement of SMN in DNA repair. DNA damage was also recorded as one of the characteristic features of the testicular cells in another mouse model of SMA [57].

5. Signal transduction

5.1. Signaling regulating the actin cytoskeleton

Neurites and growth cones are formed by Actin-rich cytoskeletal structures that undergo dynamic rearrangements in response to external and internal growth cues [226]. Given the localization of SMN to neurites and growth cones [41,227], efforts have been made to determine whether and how SMN may regulate rearrangements of these dynamic structures. SMN binds to Profilin2a, a neuron-specific Actin-binding protein, via a conserved proline-rich sequence coded by exon 5 (Fig. 1) [93,228]. SMN knockdown in PC12 cells reduces neurite outgrowth and leads to Profilin2a accumulation [229]. Rho-Associated Kinase (ROCK) regulates Profilin2a through phosphorylation (Fig. 5A) [230]. In PC12 cells with SMN knockdown, Profilin2a is hyperphosphorylated, while other downstream ROCK targets, including Myosin Light Chain Phosphatase (MLCP) and Cofilin, are hypophosphorylated (Fig. 5A) [228]. Abnormal phosphorylation of these proteins would interfere with the required actin cytoskeletal changes necessary for neurite outgrowth. Together, these findings link SMN to the RhoA/ROCK pathway. Specifically, SMN and ROCK may compete with one another for access to Profilin2a as a mechanism to regulate neurite outgrowth [228]. Dysregulation of this pathway could play a role in SMA pathology, since it leads to a pronounced effect on neuron integrity and neurodegeneration. Indeed, treating an intermediate SMA mouse model ($Smn^{2B/-}$) with the ROCK inhibitors Y-27632 or fasudil increases animal survival, although neither compound prevents motor neuron death and only Y-27632 partially ameliorates defects in neuromuscular junction (NMJ) maturation [233,234].

The Actin-binding protein known as Plastin3 adds another dimension to the role of SMN in regulation of the actin cytoskeleton. Plastin3 regulates cytoskeletal dynamics through

various mechanisms, including bundling of Actin filaments [235,236]. Interestingly, Plastin3 is a potential genetic modifier for SMA, since asymptomatic compared to symptomatic siblings with *SMN1* deletion express higher Plastin3 in lymphoblasts, but not fibroblasts [15]. Further, induced pluripotent stem cells from an asymptomatic individual differentiated into motor neurons express high Plastin3; this finding indicates that Plastin3 plays a protective role in motor neurons and specifically in growth cones [237]. SMN, Plastin3 and Actin associate in large protein complexes and Plastin3 overexpression could correct neurite outgrowth defects observed in the context of low SMN [15]. In the intermediate $Smn^{2B/-}$ mouse model, a decrease in Plastin3 levels occurs concomitantly with an increase in the Profilin2a level prior to the onset of symptoms [238]. This finding suggests that deregulation of actin dynamics precedes and is likely the mediator of motor neuron degeneration. Animal studies have produced ostensibly inconsistent results as to the benefit of Plastin3 upregulation in the context of low SMN. For example, overexpression of Plastin3 is unable to improve survival of severe SMA mice (Taiwanese and Δ7 models), although there is some benefit to NMJ formation and function [239,240]. However, when Plastin3 is overexpressed in the context of an intermediate SMA mouse model, there is marked improvement in lifespan, motor function and NMJ architecture and function [241]. Collectively, these data indicate that SMN plays an important and complicated role in regulating the actin cytoskeleton.

5.2. Signaling pathways implicated in neurodegeneration

Since degeneration of spinal cord α-motor neurons is a hallmark of SMA, studies have examined the potential role of reduced SMN on pathways implicated in neurodegeneration. One study tested activation of various Mitogen-Activated Protein Kinases (MAPKs) in human SMA and control spinal cord in an attempt to identify pathways that may contribute to neurodegeneration [231]. This screening identified activation and increased activity of c-Jun NH_2-Terminal Kinases (JNKs). Consistently, low SMN results in the JNK3 activation that contributed to the motor neuron death (Fig. 5B) [231]. While knockout of *Jnk3* in the context of the Δ7 mouse improves the phenotype, this improvement is not due to an increase in SMN protein [231]. Thus, SMN appears to act through upstream regulators of JNK3. SMN is also involved in ubiquitin homeostasis, since Ubiquitin-Like Modifying Activator 1 (Uba1) is markedly reduced in the spinal cord and the gastrocnemius muscle of severe SMA mice [232]. Consistently, restoration of UBA1 has been recently shown to ameliorate disease pathology in zebrafish and mouse models of SMA (19). Ubiquitination pathways regulate axonal and synaptic stability as well as the stability of the SMN protein [8,19,242–244]. Uba1 and SMN physically interact in the neuronal cytosol, and reduction of SMN dysregulates *Uba1* splicing, perhaps leading to the reduction of Uba1 protein [232]. Reduced Uba1 would perturb ubiquitin homeostasis and this disturbance could contribute to neurodegeneration (Fig. 5C). Interestingly, dysregulation of Uba1 is accompanied by the accumulation of β-Catenin, a normal substrate for degradation by ubiquitination. This effect appears to be tissue-specific as an increase in β-Catenin occurs only in the spinal cord but not in the heart and the liver [232]. While β-Catenin signaling has not been linked to neurodegeneration, decreased β-Catenin degradation could cause its increased translocation to the nucleus, abnormal gene transcription and subsequent neuronal instability [232]. Studies in *D. melanogaster* implicate the role of SMN in the regulation of the Fibroblast

Growth Factor (FGF) signaling pathway that controls the formation of NMJs [245]. However, the regulatory role of SMN on FGF signaling in mammalian systems has yet to be investigated. Taken together, these findings indicate that low SMN perturbs numerous signaling pathways that could contribute to neurodegeneration.

6. Intracellular trafficking, endocytosis and autophagy

6.1. Intracellular trafficking

Several studies suggest that SMN mediates neurite outgrowth through participation in intracellular trafficking of mRNA [39,40,128]. SMN localizes in granules found throughout neurites and in growth cones of cultured spinal cord motor neurons [38]. SMN, along with hnRNP R, contributes to localization of *β-Actin* mRNA to growth cones, and SMN deficiency impairs neurite outgrowth and *β-Actin* mRNA localization in growth cones [40]. More recent studies investigated the mechanism by which SMN localizes to growth cones to exert its influence [43]. COPI is a protein complex that mediates vesicular transport between the cis end of the Golgi apparatus and the endoplasmic reticulum [246]. This complex also appears to function in intracellular trafficking in neurites [43]. In axonal growth cones, SMN directly interacts with the COPI coatomer protein complex, specifically the α-COP protein, along with Gemin2 and Gemin3 proteins and *β-Actin* mRNA [43]. Depletion of α-COP in SH-SY5Y cells decreases neurite outgrowth and disrupts SMN localization at the lamellipodium, an Actin-rich dynamic structure [43]. α-COP interacts with SMN through dilysine motifs coded by exon 2b [247], and this interaction is critical for normal neurite outgrowth in PC12 cells and for motor axon development in zebrafish [248]. Further, knockdown of α-COP in NSC34 cells results in the accumulation of Smn granules in the Golgi apparatus [44]. Taken together, these results suggest that neurite outgrowth requires SMN localization to the growth cones.

When localized to growth cones, SMN could modulate neurite outgrowth through actin cytoskeleton rearrangement. In fibroblasts, SMN is recruited to the actin cortex to structures that mediate remodeling of the cytoskeleton [249]. SMN interacts with Caveolin-1, a component of caveolae in the plasma membrane, to form a translational platform that sequesters inactive ribosomes. Under appropriate cues, SMN releases these inactive ribosomes to actively translating polyribosome machinery to quickly alter the actin cytoskeleton. SMN reduction depletes the plasma membrane of inactive ribosomes and attenuates its dynamic remodeling [249]. Once localized to growth cones, SMN could stabilize these translational platforms to allow for rapid translation of *β-Actin* to allow for dynamic changes in the cytoskeleton. This potential role of SMN is attractive, since SMN mediates localization of *β-Actin* to growth cones [40]. Further research will be required to ascertain whether this mechanism occurs in neurons.

6.2. Endocytosis

Endocytosis is the de novo production of internal membranes from the plasma membrane lipid bilayer. This process internalizes integral membrane proteins, lipids and extracellular content [250]. Since endocytosis depends on actin cytoskeleton remodeling [251], it is not surprising that SMN is involved in endocytosis. In *C. elegans*, *smn-1* depletion impairs

synaptic transmission by interfering with the endocytic process of synaptic vesicle recycling [252]. Further, *smn-1* depletion causes widespread endosomal deficits, including abnormal localization of endosomal proteins, defects in endosomal trafficking in neuronal and non-neuronal tissue and impaired JC polyomavirus (JCPyV) infection, a process mediated by Clathrin-coated endocytosis [252]. In severe SMA mice (Taiwanese model), endocytosis in transversus abdominis muscle was found to be disturbed when these muscles were subjected to electrical stimulation [241]. Interestingly, overexpression of Plastin3 restores endocytosis; this finding indicates the potential phenotype-modifying capability of this gene [241]. Plastin3 also interacts with CORO1C, an Actin-binding protein implicated in endocytosis [253]; together these proteins could rescue endocytosis defects in SMN depleted cells [241]. As stated previously, SMN interacts with Caveolin-1, a major component of caveolae, and these structures can mediate endocytosis through actin cytoskeleton remodeling [250,254]. However, the exact mechanism by which SMN modulates endocytosis remains unknown.

6.3. Autophagy

Autophagy is a highly-regulated process important for normal cell growth and differentiation. In autophagy, cytosolic proteins and organelles become enclosed in double-membrane vesicles and are degraded through fusion with the lysosome [255]. Autophagy is deregulated in several neurodegenerative diseases, including ALS [256,257]. This deregulation could at least partially explain motor neuron death in SMA. Autophagy is mediated by the multifunctional signaling hub protein p62/Sequestosome-1, which recognizes both ubiquitinated proteins that are destined for degradation and the Light Chain 3 (LC3) protein in the membrane of the forming autophagosomes [255]. *Smn* knockdown in cultured mouse embryonic spinal cord motor neurons leads to accumulation of LC3-II, an indication of autophagosome formation, in the soma and neurites [258]. This increase was proposed to be attributed to an induction of autophagosome production, but not altered autophagic flux (e.g. autophagic degradation activity). However, in NSC34 cells with *Smn* knockdown, autophagic flux is compromised; specifically, the autophagosome fails to fuse with the lysosome [259]. This failure could be related to compromised intracellular trafficking correlated with an increase in the microtubule destabilizing protein Stathmin [260]. Of note, upregulated Stathmin reduces the amount of polymerized Tubulin and this disruption could compromise intracellular trafficking and interfere with fusion of the autophagosome and lysosome [259]. In severe SMA mice (Taiwanese model), there is an increase in LC3-II and p62 protein in the spinal cord as well as an increase in LC3-positive puncta during embryonic and postnatal time points [261]. Taken together, these findings suggest that SMN reduction deregulates autophagy within motor neurons, although it is unclear to which extent this deregulation contributes to motor neuron death.

7. System-wide role of SMN: Lessons learned from the animal models of SMA

Since the discovery that *SMN1* is the causative gene for SMA, much effort has gone into developing animal models of the disease. These animal models serve to not only understand the impact of low SMN on the development and function of tissues, but to allow for the testing of potential treatments for SMA. A complete description of animal models of SMA is

beyond the scope of this review. Here we briefly touch upon certain observations with implications to the specific functions of SMN. Several species serve as models for SMA, from the invertebrate nematode (*C. elegans*) and fruit fly (*D. melanogaster*) to the vertebrate zebrafish (*Danio rerio*) and mouse (*Mus musculus*) [262]. *C. elegans* and *D. melanogaster* are convenient models to examine the impact of low SMN because the endogenous *smn* is easy to manipulate, replication time of these animals is short and their development is well characterized [262]. Consistent with the critical role of the SMN N-terminus that interacts with Gemin2, p53 and nucleic acids (Fig. 1), a point mutation (D44V) at the N-terminus led to the impairment of the late-larval development and caused progressive motor function (thrashing) defects in *Caenorhabditis elegans* [263]. In case of *Drosophila*, a SMA-patient-associated point mutation (G275S) in the conserved YG-box showed NMJ defects [264]. Other point mutations in YG-box of SMN showed a wide variety of phenotypes in *Drosophila* [265]. Interestingly, the snRNP biogenesis function was found to be not a major contributor to the SMA phenotype in *Drosophila* [266]. Supporting the neuron-specific function of SMN, knockdown of SMN in zebrafish triggers defects in motor neuron outgrowth and pathfinding [267]. Validating the critical role of SMN C-terminus in conferring protein stability (7–9), truncations or point mutations in C-terminus cause NMJ defects and reduces life expectancy in zebrafish [268]. In concurrence with the findings in *Drosophila* supporting a lack of correlation between snRNP biogenesis and SMA disease pathology [266], experiments in zebrafish suggested that snRNP assembly function of SMN is not critical for rescuing the motor exon defects [101]. Overall, studies in *Drosophila* and zebrafish underscore the utility of these models in determining the impact of specific function of SMN in disease progression.

Mouse models of SMA offer a rich source to examine multi-organ effects of low SMN and to test various therapeutic strategies for SMA. Mice carry the gene *Smn*, which similar to *SMN1* predominantly includes exon 7, and as mentioned before, knockout of *Smn* is embryonic lethal [11]. Transgenic mouse models of SMA are usually generated by knocking out of the *Smn* gene coupled with the addition of various copy numbers of the *SMN2* transgene [10,12,269]. These models generally exhibit a severe phenotype with markedly reduced lifespan and are useful in evaluating early postnatal development of organ systems. A significant body of work has examined the impact of low SMN on the nervous system, especially the critical role of SMN in the maturation and function of the NMJ [45,270–272]. Collectively, these studies reveal that reduced SMN leads to the accumulation of Neurofilament protein at motor nerve plates, reduced arborization, abnormal synaptic vesicle localization, immature plaque-like NMJs and impaired neurotransmission. High SMN is required for the normal maturation of the NMJ; mice exhibit an insensitivity to reduced SMN beginning at P17, an age that correlates with maturation of NMJs [273]. The exact mechanism by which SMN influences the maturation of the NMJ remains unclear. Given the interaction of SMN with the cytoskeletal (especially actin) system [228], it is tempting to speculate that the defects could be caused by perturbed cytoskeleton regulation. In reality, the role of SMN in NMJ development and maturation likely involves multiple steps. In the Δ7 mouse, the dysregulation of synaptogenesis genes precedes the overt motor neuron pathology [274]. Notable changes include alternative splicing of Agrin, a protein crucial for NMJ maintenance, upregulation of synaptic pruning factor C1q and downregulation of the

transcription factor Etv1/ER81 [274]. The early changes in transcriptome indicate that SMN may tightly regulate the motor circuit and its reduction can impair the normal expression of relevant factors.

While SMA mouse models illustrate the importance of SMN in the nervous system [275], increasing evidence shows that SMN has a pronounced effect on tissues outside of the nervous system. SMN reduction affects the development and function of the cardiovascular system [276–278], lungs [278,279], bone [280], intestine [278,281,282], liver [283,284], pancreas [285], spleen [286] and testis [57]. Although the exact mechanism by which SMN influences the development and function of these organs is a matter of future investigation, it is clear that in addition to the nervous system the effective treatments for SMA will need to address peripheral organ defects as well. Interestingly, a recent study captured significant differences in life expectancy, muscle and NMJ pathology upon change in genetic background of a mouse model of SMA [287]. Findings of this study underscore why SMA patients display a much wider spectrum despite in many cases carrying the identical mutations. These findings also emphasize the need to understand the system-wide network of SMN interactions that are likely to vary in cells originating from different individuals.

8. Conclusions

Since the first report in 1995 that *SMN1* mutations cause SMA, tremendous progress has been made toward our understanding of SMN functions. SMN is a housekeeping protein that performs essential functions in both the cytosol and the nucleus. The multiplicity of SMN functions is rooted in the diversity of the SMN-interacting partners that associate with distinct SMN domains, including the N-terminal lysine-rich domain, the central Tudor and proline-rich domains as well as the C-terminal YG box. SMN modulates almost every aspect of RNA metabolism, including transcription, splicing, biogenesis of snRNPs, snoRNPs, telomerase, the 3′-end processing of histone mRNAs, translation, selenoprotein synthesis, stress granule formation and mRNA transport. A vast majority of SMN functions require interaction of the Tudor domain with a symmetrically dimethylated protein [79–81]. In several instances, SMN executes its functions through the formation of the multi-component RNP complexes of varied compositions. However, the specific role of SMN in most of these complexes remains unknown. SMN harbors a distinct nucleic-acid-binding domain that shows preference for G-rich sequences in vitro [73]. SMN also interacts with RNA-binding proteins that are involved in trafficking of mRNAs within motor neurons. Future studies will determine if a direct interaction between SMN and RNAs is the driving force behind the formation of various RNP complexes.

Independent of its role in RNA metabolism, SMN regulates other functions, including but not limited to DNA repair, cell signaling, endocytosis, autophagy and the neuronal cytoskeleton. Early death of motor neurons in severe SMA triggers a series of events common to several neurodegenerative diseases. It is likely that low levels of SMN in motor neurons simultaneously impact multiple functions. Peripheral defects in mild SMA point to the intrinsic need for SMN in all tissues. Studies on disease-modifying factors of SMA suggest that the impact of low levels of SMN could be partially mitigated but not fully compensated. This suggestion is consistent with the involvement of SMN in key cellular

processes, which require high precision and fine-tuning. Based on the mislocalization of SMN and/or perturbations in SMN-associated functions, the role of SMN has been implicated in inclusion-body myositis and ALS [52,53]. In the case of osteoarthritis, SMN is expressed at an aberrantly high level in cartilage [54]. On the other hand, low SMN expression has been recently linked to testicular defects and male infertility [57]. These results support that both aberrantly low and high SMN expression could result in pathological conditions. The number of SMN-associated pathologies is likely to grow based on the diverse nature of interactions forged by SMN. Considering its involvement in upstream events such as transcription, splicing, mRNA trafficking and translation, SMN has the potential to regulate its own expression. Our understanding of SMN functions will continue to improve as we acquire more knowledge of the mechanism of various cellular processes. With a better understanding of SMN functions, we will uncover novel disease mechanisms, which will bring us closer to effective and targeted therapies for SMA and other related diseases.

Acknowledgments

Authors acknowledge Dr. Brian Lee for providing computer generated model of SMN protein. Authors have attempted to include most contributions on SMN functions and have provided references to review articles on specific topics. Authors acknowledge and regret for not being able to include all the references due to the lack of space.

Funding: This work was supported by grants from the National Institutes of Health (R01 NS055925, R21 NS072259 and R21 NS080294), Iowa Center for Advanced Neurotoxicology (ICAN), and Salsbury Endowment (Iowa State University, Ames, IA, USA) to RNS.

Abbreviations

SMA	spinal muscular atrophy
ALS	amyotrophic lateral sclerosis
SMN	Survival Motor Neuron
RNP	ribonucleoprotein
hnRNP	heteronuclear RNP
snRNA	small nuclear RNA
snoRNA	small nucleolar RNA
rRNA	ribosomal RNA
snRNP	small nuclear RNP
snoRNP	small nucleolar RNP
TERC	Telomerase RNA component (TERC)
TERT	Telomerase Reverse Transcriptase
TGS1	Trimethylguanosine Synthase 1, TMG, 2,2,7-trimethylguanosine

SG	Stress granule
CG	Cajal body
iCDR	Centromeric Damage Response
NMJ	Neuromuscular junction
TCR	transcription-coupled repair
CBP20	Cap-Binding Protein 20
CBP80	Cap-Binding Protein 80
EWS	Ewing's Sarcoma Protein
FMRP	Fragile X Mental Retardation Protein
PHAX	Phosphorylated Adaptor for RNA Export
Sec	Selenocysteine
Secis	Sec insertion sequence
SBP2	Secis-Binding Protein 2
TIA1	T-cell Restricted Intracellular Antigen 1
WRAP53	WD40 Repeat-Containing Protein Encoding RNA Antisense to p53
Xpo1	Exportin 1

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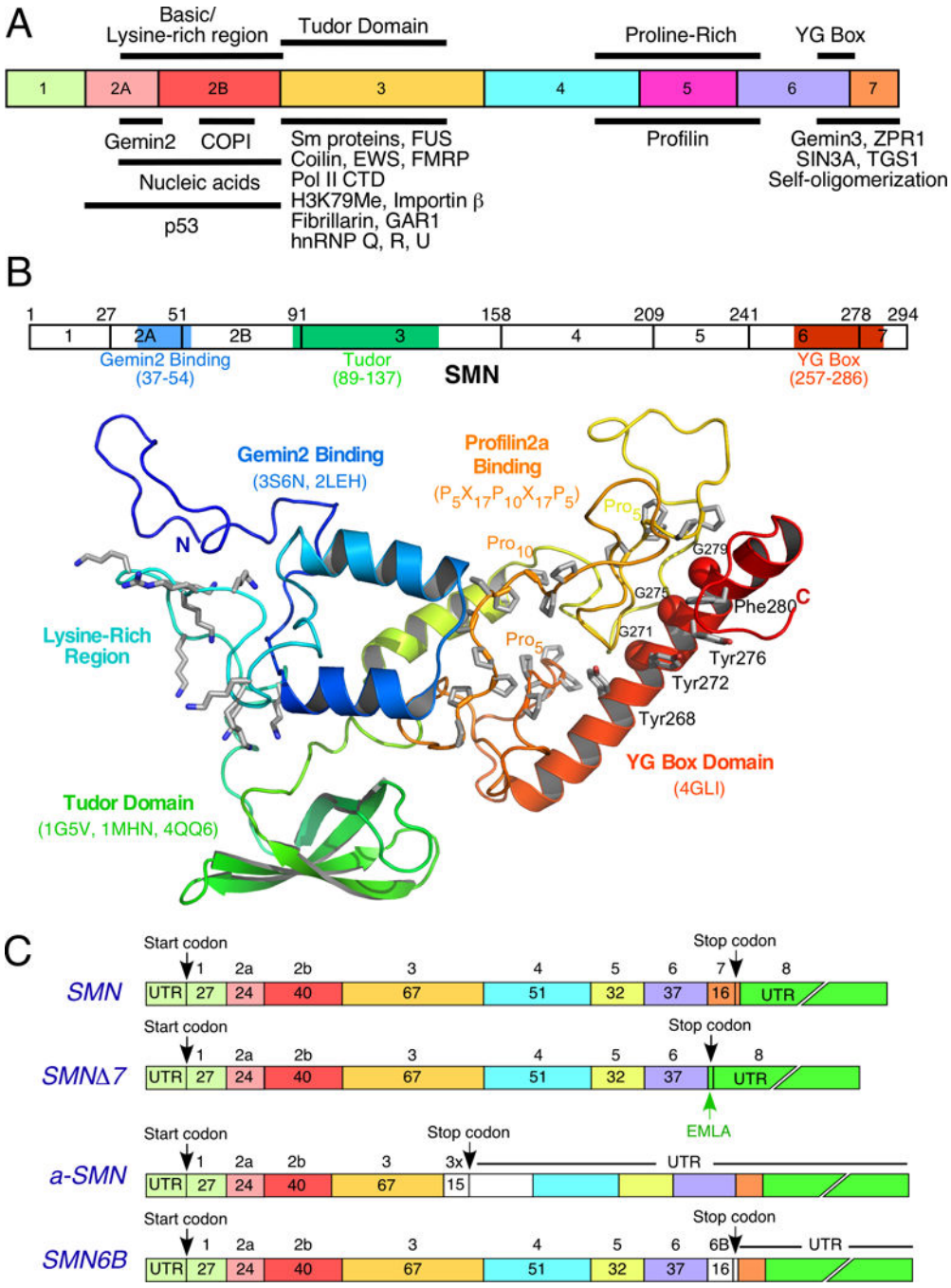

Figure 1. Structure of SMN protein and *SMN* transcripts

(A) Diagrammatic representation of the SMN protein. The numbers in the colored box indicate the exon. Domains are indicated above the boxes and proteins shown to interact with SMN are shown below. See text for further details about proteins. (B) Comparative modeling of the full-length SMN protein utilized multiple structure templates for the Gemin2 binding domain (blue) [20,21], the Tudor domain (green) [22–24] and the YG Box domain (orange) [25]. Model calculations with RosettaCM included the structure templates for the domains and fragment libraries derived from sequence-based searches of the Protein

Data Bank for modeling all other regions [26]. The SMN protein diagram shows the structural domains in color overlaid on a map of the labeled exons with the number of the last amino acid residue in each exon indicated above. The structural domains and amino ranges are indicated below the diagram. The full-length SMN protein model is shown as a cartoon representation with a rainbow color scheme from blue N-terminus to red C-terminus (labeled N and C, respectively) [27]. The structured domains are indicated with the PDB codes of the comparative modeling templates listed below each name. The unstructured regions highlighted include the lysine-rich region, and the Profilin2a binding region with the conserved proline-rich sequence indicated below the label. Selected amino acid side chains are shown as stick representations with blue for lysine amino groups and red for tyrosine hydroxyl groups. The conserved residues of the YG Box motif are indicated with the Cα atoms of the glycine residues shown a van der Waals spheres. (C) Diagrammatic representation of transcripts generated from *SMN*. The name of each transcript is indicated to the left. Start and stop codons are indicated for each transcript, the exon number is indicated above the colored boxes and the number of amino acids coded by each exon is indicated in the boxes. The EMLA degron [9] that renders *SMNΔ7* unstable is indicated. Abbreviation: UTR, untranslated.

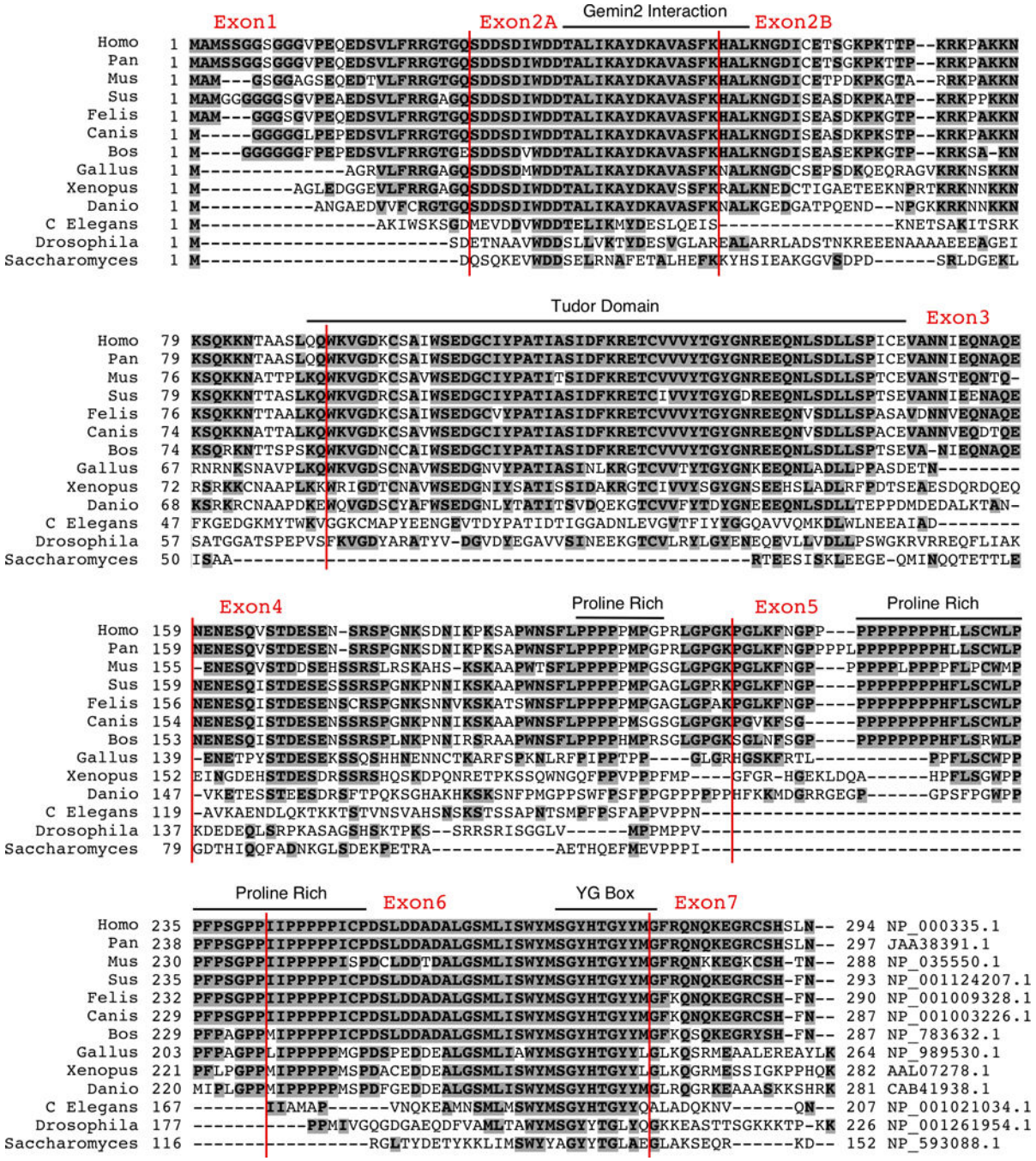

Figure 2. Alignment of SMN protein from various animal species

Exon numbers correspond to human *SMN* exons. Sequences were aligned with the ClustalW algorithm using MacVector software. The name of each species is indicated to the left of the sequence and accession numbers are indicated at the end of the sequence. Bold letters highlighted in grey indicate consensus amino acids. The highly conserved Gemin2 interaction, Tudor domain, polyproline-rich domain YG Box are denoted. Adapted from [50].

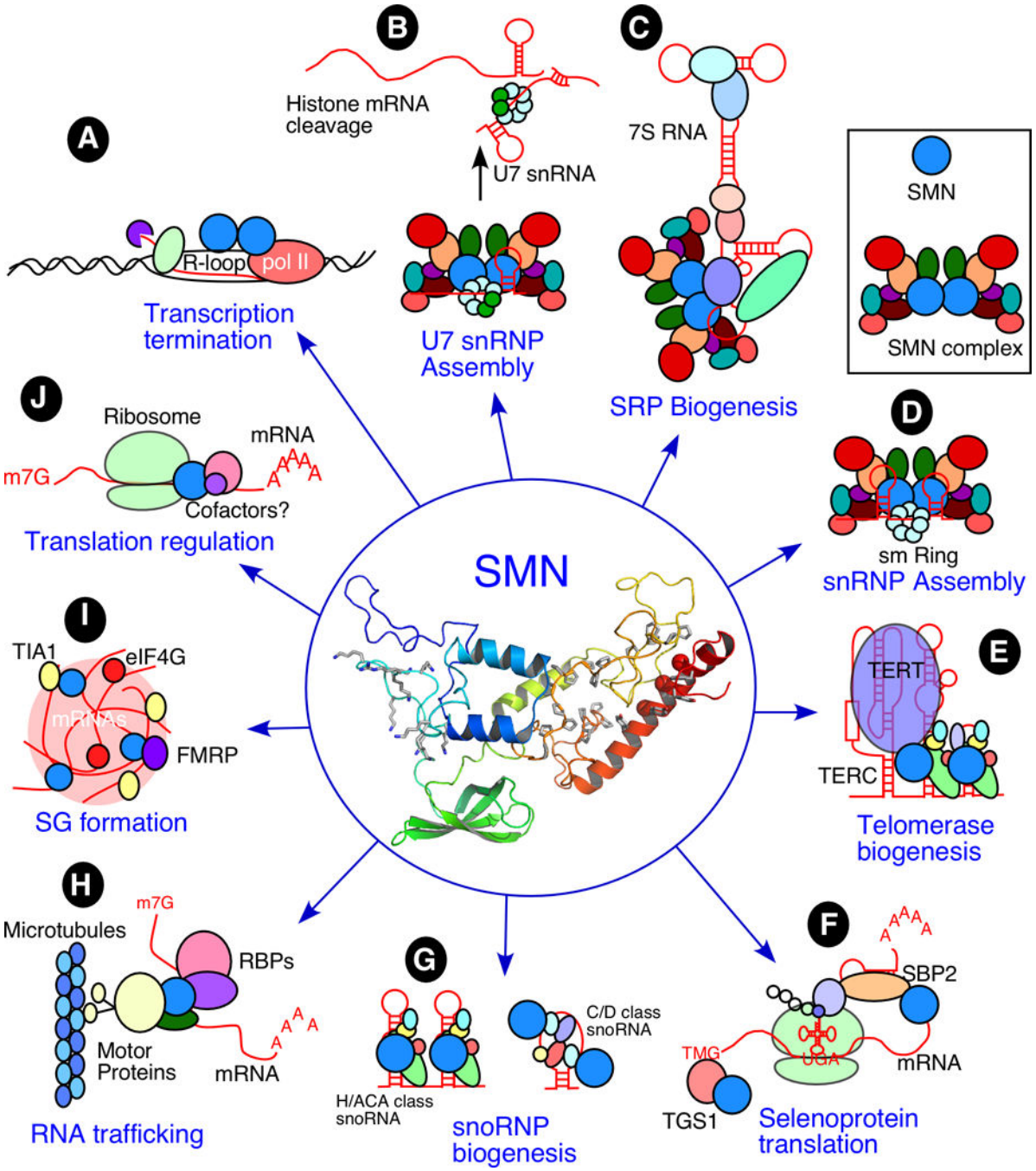

Figure 3. Proposed RNA metabolism roles of SMN

SMN is pictured at the center and is represented in all cases by a blue circle. All RNAs are represented by red line drawings, all DNA in black, and all proteins as colored ovals or circles. Starting from the top left, in clockwise order, the functions are as follows. (A) Transcription termination: SMN interacts with symmetrically dimethylated arginines in the C-terminal domain CTD of polymerase II, and is proposed to assist in targeting the Senataxin helicase to R-loops [90]. (B) U7 snRNP assembly: The SMN complex loads the Sm/Lsm ring onto U7 snRNA [116,117]. Afterwards, the mature snRNP functions in 3′ end

processing of histone mRNAs [117]. (C) SRP biogenesis: the SMN complex is required for proper interaction between SRP54 and the 7S RNA, which is required for targeting of nascent polypeptides to the endoplasmic reticulum [123]. SRP54 is pictured as a purple oval. (D) Spliceosomal snRNP assembly: SMN functions along with the other components of the SMN complex [20,72,108–115] in assembly of the heptameric Sm ring onto snRNA [109]. After assembly, mature snRNPs catalyze pre-mRNA splicing in the nucleus. (E) Telomerase biogenesis: Functional telomerase contains an RNA component (TERC) as well as several proteins. SMN interacts with telomerase-associated proteins GAR1, TERT, Dyskerin, and WRAP53, and is proposed to function in targeting telomerase to Cajal bodies (CBs) [82,107,119]. (F) Selenoprotein translation: SBP2 causes incorporation of selenocysteine (Sec) in the place of a stop codon. In addition, many selenoprotein mRNA 5′ ends are hypermethylated by TGS1 [100]. SMN interacts with both SBP2 and TGS1 [100]. (G) snoRNP biogenesis: H/ACA and C/D class snoRNPs consist of RNA and a characteristic set of protein cofactors for each class. SMN interacts with Fibrillarin, a component of C/D class snoRNPs [118], and GAR1 and Dyskerin within H/ACA class snoRNPs [82,107]. (H) RNA trafficking: SMN interacts with a number of RNA binding proteins known to assist in targeting of mRNAs to axon terminals [87,124–129], and appears to actively participate in the process [40,127–130]. (I) Stress granule formation: SMN is present in stress granules (SGs) and low levels of SMN impair the formation of SGs [46,120]. (J) Translation regulation: SMN regulates the level of CARM1 protein [121] through a translation-dependent mechanism proposed to involve a direct interaction between SMN and polysomes [122].

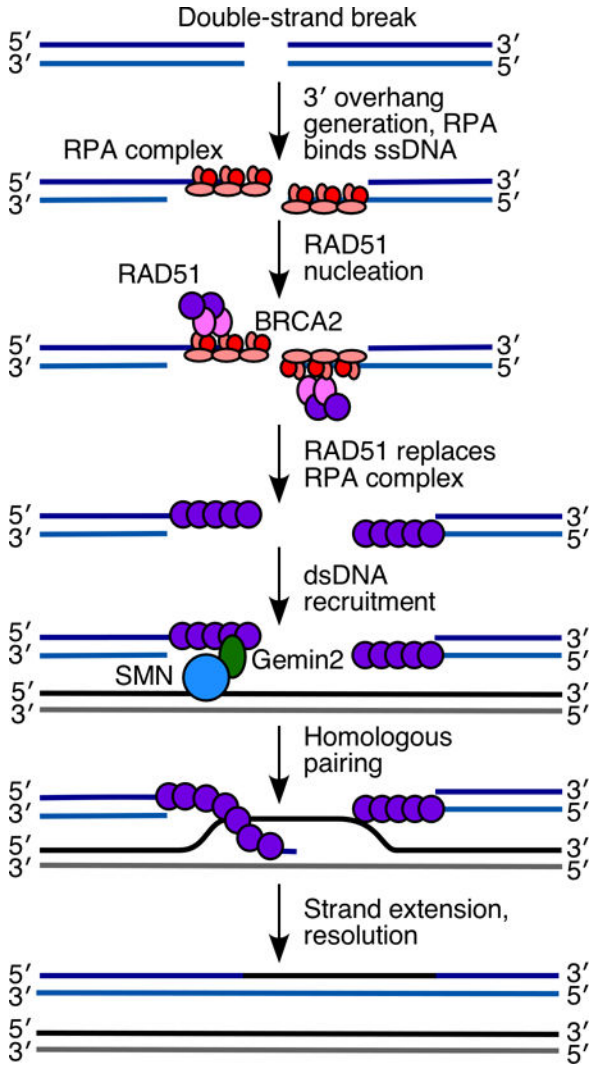

Figure 4. Proposed function of SMN in double-strand break repair

Double-strand DNA break repair is a multi-step process that involves replication of DNA from a homologous chromosome. First, one strand from each end is digested by exonucleases and the newly generated single-stranded DNA (ssDNA) region is bound by the RPA complex. Next, BRCA2 oligomers are recruited to RPA-bound ssDNA and help to nucleate RAD51 oligomerization on the ssDNA. RAD51 then continues to oligomerize and completely replaces RPA on the ssDNA. RAD51-coated ssDNA then attempts to pair with homologous regions of double-stranded DNA (dsDNA). A SMN-Gemin2 fusion protein increases the association of RAD51-ssDNA with heterologous dsDNA in vitro [222], and so is proposed to function at this stage. Once a homologous region of dsDNA is identified, it is invaded by the ssDNA and strand extension takes place. At this stage, there are multiple outcomes, depending on whether there is crossover between the homologous chromosomes and whether both ends of the break are extended. For brevity's sake, the simplest outcome, synthesis dependent strand annealing, in which a single strand is extended and then re-anneals with its complementary strand, is portrayed here. For a more detailed overview of double-stranded break repair, see Godin et al 2016 [219].

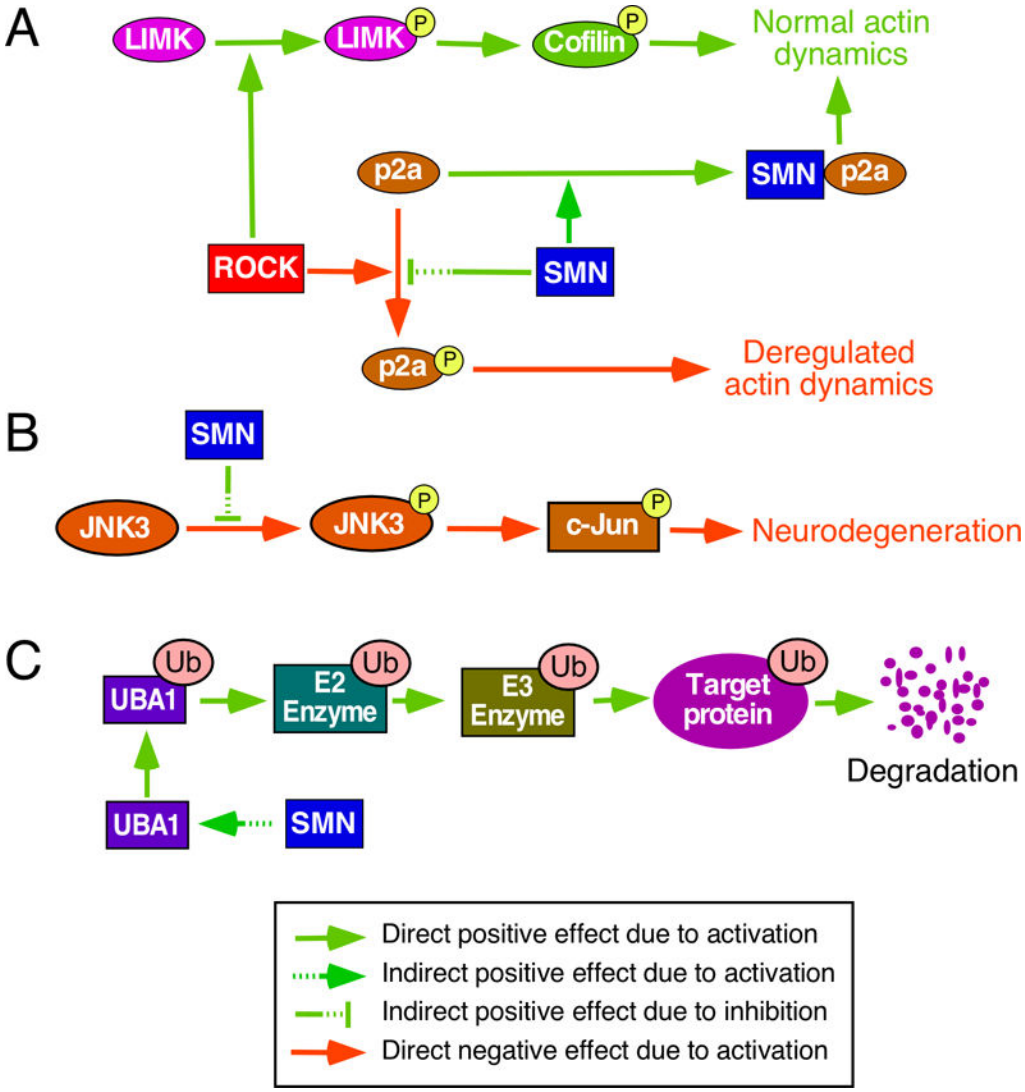

Figure 5. SMN involvement in signaling pathways

(A) SMN involvement in regulation of actin dynamics. Rho-Associated Kinase (ROCK) phosphorylates Lim Kinase (LIMK) which in turn phosphorylates Cofilin (denoted by P in yellow circle) to contribute to actin dynamics regulation. SMN can prevent ROCK-mediated phosphorylation of Profilin2a (p2a) by interacting and forming a complex with p2a. When SMN is reduced, the interaction between p2a and ROCK increases, with a concomitant increase in p2a phosphorylation and a decrease in LIMK and Cofilin phosphorylation. Consequently, actin dynamics are deregulated and this leads to neurite outgrowth inhibition and neurodegeneration [228]. (B) SMN regulates the activation of c-Jun NH_2-Terminal Kinases 3 (JNK3) by an unknown mechanism. When SMN is reduced, there is an increase in phosphorylation and thus activation of JNK3 and a subsequent increase in c-Jun phosphorylation. This increased activation can lead to neurodegeneration [231]. (C) SMN binds to and can regulate the level of Ubiquitin-Like Modifier Activating Enzyme 1 (UBA1; denoted by the dashed arrow). UBA1 can subsequently initiate the ubiquitination pathway

that leads to degradation of target proteins. A reduction in UBA1 alters ubiquitin homeostasis and may lead to neurodegeneration [232].